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A.M.A. ARCHIVES OF
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Myocardial Infarction in Women

A Study of Autopsy Populations

FAIRFIELD GOODALE, M.D., Hanover, N.H.; WILBUR A. THOMAS, M.D., and ROBERT M. O'NEAL, M.D., St. Louis

Introduction

Scarcely any medical dictum concerning arteriosclerosis and its complications is as deeply entrenched as that relating to the great prevalence of acute myocardial infarction in men as opposed to women. Cecil and Loeb's "Textbook of Medicine" [1] states that "acute myocardial infarction affects men approximately six times as often as women," and Robbins' recently published text in Pathology [2] gives the figures as 3:1. Many articles support this view.[3-6] A recent study failed to demonstrate this difference in sex incidence in the autopsies performed at Washington University in the years 1940-1954.[7] Other recent autopsy studies also indicate that the supposedly greater susceptibility of males than females to myocardial infarction must be reevaluated. The ratio of males to females with healed infarcts in the series of Gould and Cawley,[8] in Detroit, was 1.13:1. The results of some [9] differ from the Washington University study, although the data are not directly comparable.

In order to establish more accurately the actual prevalence of acute myocardial infarction at autopsy, especially its proportionate frequency in the two sexes, this study has more than tripled the volume of data by adding to the analysis of autopsies from Washington University those of two other large series from widely separated areas: one from the Massachusetts General Hospital in Boston, and the other from the Radcliffe Infirmary in Oxford, England. The results confirm that the incidence of acute myocardial infarction in autopsy populations is now similar in the two sexes. Such results are difficult to reconcile with the unshakable faith held by clinicians that the disease is conspicuously commoner in men than in women. Likewise, the results conflict with the reported death rate from "arteriosclerotic heart disease," which is materially higher in men than in women (ratio 2.2:1 in 1955 among whites [10]), as derived from certifications of death in the general population of the United States. The lack of agreement between the data derived from autopsies and those obtained from certifications of death is not difficult

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We are grateful to Dr. A. H. T. Robb-Smith for permission to examine the autopsy files of the Radcliffe Infirmary, Oxford, England.

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Part of this work was performed during the tenure of a U.S. Public Health Service Research Fellowship (HF-5174-C) of the National Heart Institute. (Dr. Goodale).

From the James Homer Wright Pathology Laboratories, Massachusetts General Hospital, Boston; the Department of the Reguis Professor of Medicine, Radcliffe Infirmary, Oxford, England, and the Department of Pathology, Washington University School of Medicine, St. Louis.

to understand. Autopsies are performed on only a small fraction of patients dying, whereas certification of death must be made out for all. Even with modern diagnostic methods, the vast majority of completed certifications of death with the primary cause as "acute coronary thrombosis" or "acute myocardial infarction, are, at best, educated guesses. Autopsies provide objective, accurate information regarding the cause of death.

However, it must be emphasized that data derived from hospital populations or autopsies cannot be used directly in drawing conclusions regarding the population of a geographic area, as unknown factors of selection undoubtedly exist.

Material and Methods

Autopsies.—The autopsy files in all three departments of pathology were examined. Only adults (over 20 years of age) were included in the study. A total of 13,485 adult autopsies were reviewed: 4,436 performed at Washington University (1940-1954), 5,460 from the Massachusetts General Hospital (1945-1954), and 3,589 from the Radcliffe Infirmary (1946-1953). The average ages of the patients at these three hospitals were 56 (men 57, women 53), 61 (men 61, women 60), and 58 (same for men and women) years, respectively. From Washington University only white patients were included. Figures as to race from the Massachusetts General Hospital and Radcliffe Infirmary were not accurately compiled, but very few Negroes are autopsied at either institution. All patients were classified as to age, sex, and presence or absence of acute myocardial infarcts, defined as having an estimated duration of one month or less. This strict anatomic definition requires that muscle necrosis and inflammatory cellular reaction be present; therefore, the very old and very early infarcts in which the anatomic diagnosis cannot be made with certainty are eliminated.

Clinical Records.—The study of clinical records was confined to those of Barnes Hospital (Washington University). The first four clinical diagnoses of patients have been coded and are readily available in "IBM" books[11] for the years 1942-1954. All the books were examined and data from the following four groups of patients tabulated according to age and sex: (1) all adults who died in the hospital; (2) all adults who died and had a clinical diagnosis of myocardial infarction; (3) all adults who had a clinical diagnosis of myocardial infarction and were discharged alive; (4) a random sample consisting of 3 out of each 200 patients discharged alive during the entire period.

Results

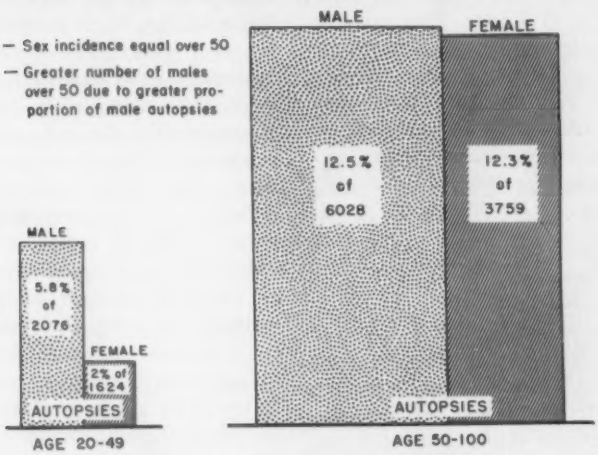
Autopsies.—The results of the autopsy analysis are presented in Table 1. Of the 9,785 patients over 50 years of age, no statistically significant difference was found between the numbers of acute myocardial infarct in men and the numbers in women (ratio 1.02:1). Among the 3,700 patients less than 50 years of age, a highly significant sex difference was found (ratio 2.87:1, male to female).

Of the total 13,485 patients, 1,372 (10.2%) had acute myocardial infarcts

TABLE 1.—*Incidence of Acute Myocardial Infarcts (AMI) Among 13,485 Patients Autopsied at Massachusetts General Hospital, Boston; Radcliffe Infirmary, Oxford, England, and Washington University, St. Louis 1945-1954*

		Men			Women				
Hospital	Age Group	Total No.	With AMI	Per Cent	Total No.	With AMI	Per Cent	Ratio of Sex Incidences	*P* Value for Sex Differences
M.G.H.	20-49	657	56	8.52	601	10	1.66	5.31:1.00	$P<0.01$
M.G.H.	50-100	2,459	398	16.18	1,733	264	15.23	1.06:1.00	$P>0.05$
M.G.H.	All 20-100	3,126	454	14.50	2,334	274	11.70	1.24:1.00	$P<0.01$
R.I.	20-49	573	23	4.01	383	8	2.08	1.93:1.00	$P>0.05$
R.I.	50-100	1,602	135	8.41	1,031	76	7.37	1.14:1.00	$P>0.05$
R.I.	All 20-100	2,175	158	7.26	1,414	84	5.94	1.22:1.00	$P>0.05$
W.U.	20-49	846	42	4.96	640	15	2.34	2.12:1.00	$P<0.01$
W.U.	50-100	1,955	222	11.35	995	123	12.36	0.92:1.00	$P>0.05$
W.U.	All 20-100	2,801	264	9.42	1,635	138	8.44	1.12:1.00	$P>0.05$
Total	20-49	2,076	121	5.83	1,624	33	2.03	2.87:1.00	$P<0.01$
Total	50-100	6,026	755	12.50	3,759	463	12.30	1.02:1.00	$P>0.05$
Total	All 20-100	8,102	876	10.81	5,383	496	9.21	1.17:1.00	$P<0.01$

Incidence of Acute Myocardial Infarction by Age and Sex Among 13,487 Adults Autopsied at the Massachusetts General Hospital, Boston, Radcliffe Infirmary, Oxford, and Barnes Hospital, St. Louis.



The HEIGHTS of the bars represent the percentage incidences and the AREAS of the bars represent the the actual number of patients with Acute Myocardial Infarction in each category.

demonstrated at autopsy, but only 154 of the infarcts occurred in patients under 50 years of age (Figure).

As can be seen from Table 1, the frequency of acute myocardial infarction was highest at the Massachusetts General Hospital and lowest at the Radcliffe Infirmary. Essentially equal sex ratios were observed among the autopsied patients at each of the three hospitals.

Deaths with Clinical Diagnosis of Acute Myocardial Infarction.—These data are presented in Table 2 to show their similarity to those derived from autopsies. In the period 1942-1954, the number of deaths occurring in Barnes Hospital was 5,431, of

TABLE 2.—*Sex Incidence of Acute Myocardial Infarction Among Patients at Washington University: A Comparison of Autopsy and Clinical Data*

		Autopsies			Deaths with Clinical Diagnoses Only			Discharged Alive with Clinical Diagnoses		
		Total	With AMI	Per Cent	Total	With AMI	Per Cent	Total *	With AMI	Per Cent
Adults 20-49	Men	846	42	4.96	870	47	5.4	38,220	256	0.66
	Women	640	15	2.34	760	19	2.5	48,510	27	0.055
	Ratio men:women		2.12:1			2.2:1			12:1	
Adults over 50	Men	1,955	222	11.35	2,318	357	15.4	38,220	898	2.34
	Women	905	123	12.36	1,483	215	14.5	31,640	315	0.99
	Ratio men:women		0.92:1			1.1:1			2.4:1	
Total	Men	2,801	264	9.42	3,188	404	12.6	76,440	1,154	1.51
	Women	1,635	138	8.44	2,243	234	10.4	80,150	342	0.43
	Ratio men:women		1.12:1			1.2:1			3.5:1	

* The figures in this column are projected from a sample as described in the text.

whom 59% were of men and 41% were women. Of the 1,630 patients under 50 years of age, 4% had a clinical diagnosis of acute myocardial infarction (men 5.4%, women 2.5%; ratio 2.2:1). This same diagnosis was made in 15% of the 3,801 patients over 50 years of age (men 15.4%, women 14.5%; ratio 1.1:1). Of the total 5,431 patients, 638 had this diagnosis (men 12.6%, women 10.4%; ratio 1.2:1).

Patients Discharged Alive with Clinical Diagnosis of Acute Myocardial Infarction.

These data are presented in Table 2 to show their dissimilarity to those derived from autopsies and deaths with clinical diagnoses. In the period 1942-1954, a total of 156,590 patients were discharged from Barnes Hospital. A sample of 3 patients from each 210 discharges indicates that 49% of all patients discharged were men and 51% were women; men were equally divided above and below the age of 50, but 59% of the women were under 50 years of age. In this period a clinical diagnosis of acute myocardial infarction was recorded for 1,496 patients discharged from the hospital alive (0.95% of total discharges). Of the 86,730 patients under 50 years of age, 0.33% had clinically diagnosed acute myocardial infarction (men 0.66%, women 0.055%; ratio 12:1). Of the 69,860 patients over 50 years of age, 1.73% of the patients who were discharged alive had the clinical diagnosis of acute myocardial infarction (men 2.34%, women 0.99%; ratio 2.4:1).

Comment

In a previous study we pointed out that in the autopsy series at Washington University the sex ratio of incidence of acute myocardial infarction had gradually changed over the last 30 years, so that the disease, once predominantly affecting men, was as common in women as in men during the period 1940-1954. Because of the undoubted selection of patients that occurs in any single medical center, the study was extended to two other institutions, confirming the results of the initial study. It would appear that whatever factors of selection exist at one institution also exist at the other two. The sex ratio of acute myocardial infarction found at autopsy has changed in recent years,[7] and at present (1945-1954), according to the data presented herein from three large medical centers widely separated geographically, the only difference in sex incidence is found below the age of 50 years. Even in this younger age group, the difference in incidence between men and women (2.87:1) is less than the over-all difference in incidence for all age groups as given in standard medical texts. The incidence of acute myocardial infarction is equal in men and in women past 50 years of age among autopsied patients included in this study. That the data from each of the three medical centers agree with the others would suggest that the findings from Barnes Hospital, published previously, are representative of a general trend in the changing epidemiologic characteristics of acute myocardial infarction in autopsy series.

The clinical experiences at Barnes Hospital in the diagnoses of acute myocardial infarction, as recorded in IBM diagnosis books, were similar to the autopsy findings when comparable groups (fatal cases) were considered. Clinical diagnoses of acute myocardial infarction were in the same sex ratio among patients dying in the hospital as were the anatomic diagnoses at autopsy. However, deaths in the hospital were predominantly among males (59%), similar to the predominance of men among our autopsied patients (63%). A hospital clinician or pathologist not aware of the predominance of men among his patients might easily be misled in his estimate of the sex incidence of a disease by the absolute number of men seen in his selected practice. A predominance of men is almost always present in an autopsy series,[12] but in the present study was also found to be present among the hospital discharges in all groups except that of patients below the age of 50 discharged alive (Table 1).

The only striking difference in the incidence of acute myocardial infarction between the two sexes is found in the patients discharged alive (Table 2). In this group the over-all incidence in men was 3.5 times that in women; but when only patients under 50 years of age were considered, the ratio of men to women was 12:1, a startling difference in comparison with that found among the autopsied patients in the same age group (2.12:1), as well as among patients discharged dead with only a clinical diagnosis (2.2:1). This remarkable preponderance of men discharged alive with clinically diagnosed acute myocardial infarction, six times as great a ratio as that found in autopsied patients in the same age group, suggests at least two possibilities: (1) The disease is more likely to be fatal in women, or (2) the diagnosis is less obvious in young women than in men, perhaps solely because the possibility of the diagnosis is not often entertained in young women. That the first explanation is unlikely is suggested by the equal incidences of healed infarcts found in the two sexes in a previous study,[13] as well as the study of Gould and Cawley.[8] The second possible explanation is more likely correct, for it seems reasonable to speculate, on the basis of our results, that there exists a greater clinical suspicion of this disease in men than in women; this factor would be most likely to operate when the disease is mild, nonfatal, and therefore difficult to diagnose. If this second explanation is correct, it is important that the similar incidence of acute myocardial infarction in men and in women be recognized, for the data presented herein must then indicate that many women with acute myocardial infarcts have not had the benefit of an accurate clinical diagnosis, or that the diagnosis is being made too frequently in men, or both.

It must be reemphasized that data derived from hospital or autopsy populations cannot be used directly in drawing conclusions concerning the population of a geographic area. Studies, such as that being conducted in Framingham, Mass.,[14] should eventually serve to clarify many of the epidemiologic features of acute myocardial infarction if autopsies are performed as the patients being studied die. Unless such studies are based on autopsy diagnoses, the results will be open to question.

Summary

According to data obtained from three large medical centers, widely separated geographically, the autopsy incidence of fatal acute myocardial infarction is now similar in men and in women, except for the small percentage of infarcts occurring in patients under 50 years of age. These data from autopsies agree with those derived from clinical diagnoses of patients dying at Barnes Hospital in St. Louis. However, among those patients discharged alive, acute myocardial infarction was diagnosed clinically in men much more frequently than in women; this finding is perhaps the result of a lack of clinical suspicion of the disease in women, affecting the accuracy of clinical diagnoses in mild cases.

Great caution is necessary in using data derived from autopsies for drawing conclusions regarding the general population because immeasurable factors of selection are present. However, the accuracy of diagnoses made at autopsy as compared with clinical diagnoses derived from certifications of death must be taken into account.

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Experimental Applications of Bone Marrow Transplantation

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The transplantation replacement of the hematopoietic system after destruction by irradiation or chemical injury is one of the striking achievements in experimental biology and medicine. Furthermore, the transplantation or repopulation is usually accomplished in a unique manner by injecting living donor blood-forming cells into the blood stream of the injured recipient. Some of these injected cells localize in the destroyed bone marrow. Their proliferation gives rise to a new organ system showing normal physiologic activities.

In this report we shall first outline briefly the important variables that must be taken into account for successful bone marrow transplantation in irradiated animals. We shall then discuss the experimental therapeutic applications resulting from ideas and techniques suggested, in most instances, by the studies on bone marrow transplantation. These experimental applications in animals are grouped to show how bone marrow transplantation can be used to treat chemical injury to bone marrow or replace genetically defective (hereditary anemia) bone marrow. Another section covers the use of bone marrow transplantation as an aid in homografting of skin, ovaries, or other tissues. A special group of experimental applications is the use of bone marrow transplantation (1) as auxiliary treatment of animals with advanced or disseminated cancer and (2) in the prevention of induced and spontaneous leukemia in mice.

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From the Biology Division, Oak Ridge National Laboratory; operated by Union Carbide Corporation for the U.S. Atomic Energy Commission.

Our third objective is to demonstrate how bone marrow transplantation studies have contributed to progress in immunology and immunogenetics. Finally, we wish to point out that even more basic studies on systems seemingly far removed from mammals must be continued and expanded to lay the groundwork for further experiments on the control of special disease and injury problems in man.

The Bone Marrow Experiment

The techniques in procuring and preparing a suspension of living bone marrow cells for intravenous injection in small laboratory rodents are fairly simple and easily carried out. In large animals, such as the dog, monkey, and man, procurement and preparation of the marrow present a much greater problem. The details of procurement and preparation methods investigated so far are given in the reports on large animal and human experiments (bibliography compiled by Fortuine and Baxter [1] and that by Mathé [2] for reference to most work mentioned in this paper).

The mechanism of recovery of lethally irradiated animals given an injection of bone marrow was discovered by a number of different investigators in several laboratories.[1,2]

Bone marrow cells given intravenously to the irradiated animal lodge in the damaged marrow spaces and repopulate the blood-forming organs. New peripheral blood elements are produced that prevent infection, hemorrhage, and anemia, which are the major causes of death in the radiation-exposure range under consideration. Where the donor marrow is not genetically identi-

cal (homologous and heterologous relation) with that of the host, injury to the lymphatic system is essential for therapeutic action of the marrow, since an intact or a partially intact immune mechanism in the host would reject the donor cells.

There are special donor-host genetic situations in inbred animals (isologous relation) where lymphatic tissue damage and injury to the immune mechanism are not essential for marrow transplantation; in these, experiments can be done at low as well as at the optimal radiation exposures. A similar favorable genetic situation exists between identical twins in man, and, of course, autologous bone marrow transplantation requires only the technical considerations of proper procurement and handling of marrow to get successful takes.

One of the most important variables in homologous and heterologous bone marrow transplantation is the radiation dose. In several species, radiation exposure in the vicinity of 1,000 r seems to be optimal for getting sufficient lymphatic tissue damage to permit transplantation of foreign bone marrow. At very low radiation exposures foreign marrows do not transplant well enough to be of any value to the injured animal, and at exposures well above 1,000 r the animal dies of intestinal damage before the bone marrow therapy can be of any benefit. There is, however, an intermediate range, somewhat below 1,000 r, where temporary bone marrow transplantation can also prevent radiation death. In rather special circumstances the foreign bone marrow transplantation can even make the acute radiation syndrome worse.

Another important variable in bone marrow transplantation, regardless of the donor-host genetic relationship, is the amount of marrow injected. In general, the more marrow injected the more quickly the transplantation takes place, and the peripheral blood elements are depressed for the shortest period of time.

A special phase of bone marrow transplantation in animals, particularly mice, deals with the immunologic complications that arise when a foreign marrow is established and functioning in an irradiated host. A new disease syndrome appears that we have called the "foreign-bone-marrow reaction." Its pathogenesis depends on genetic incompatibility between the donor and the host tissues. Clinically, we think of the disease as a type of "metabolic starvation," since the animals lose weight, though their food intake is normal, and at autopsy, among other changes, show a picture compatible with a starvation death. A certain percentage of foreign-bone-marrow-treated animals recover from the disease, and there is some evidence that homologous fetal blood-forming tissues cause a great deal less immunologic difficulty.

Many other variables in the bone marrow experiment have been studied. These include route and time of administration, kinds of hematopoietic tissue, preservation and tissue culture of marrow, and type and intensity of radiation.

Late effects of whole-body irradiation, as far as we can now tell, are only partially influenced by bone marrow therapy under the most favorable genetic circumstances. This partial effect is limited to prevention of thymic lymphosarcoma in irradiated-shielded or bone-marrow-treated mice, and possibly the prevention of myeloid leukemia in shielded mice. Shielding marrow or other blood-forming cells during irradiation is generally thought to have the same effect as autologous bone marrow injection. Total life span is shortened by whole-body exposure, and bone marrow therapy does not seem to have much effect on this aspect of the late effects of ionizing irradiation.

Chemical Damage to Bone Marrow

Weston and his associates studied chemical damage to bone marrow in rats. Death from lethal doses of bisulfan (Myleran) could be prevented by intravenous injection of living rat bone marrow cells within a few days after chemical injury. Further studies on bone marrow treatment of rats after chemical injury were made by other groups. Chemical damage to mice has also

been treated with bone marrow therapy. In most cases it is not established that the injected bone marrow forms transplants, but this mechanism is presumed to account for survival after lethal doses of a chemical. In situations where definitely homologous bone marrow was effective, we have assumed without further evidence that the chemical also injured the immune mechanism. Treatment of chemical damage to blood-forming tissues by bone marrow transplantation, whether autologous or homologous, is a problem of major interest, since we would like to test radical chemotherapy in cancer treatment. McFarland, Granville, and Dameshek [3] report studies on cancer patients treated by chemotherapy and autologous bone marrow.

Treatment of Hereditary Anemia

The experiments by Elizabeth Russell and her colleagues at Bar Harbor, Maine, on the cure of a hereditary anemia in mice by use of small radiation exposures to the total body and normal isologous bone marrow injection is the only experiment of this type yet to be reported. They could not use normal homologous bone marrow because the animals would not tolerate total-body irradiation in high enough doses to make homotransplantation feasible. In general, it seems that a relatively high (~900 r) x-ray exposure is necessary in many species to get sufficient damage to the immune mechanism for homologous organ system replacement.

Ceppellini [4] has suggested a bone marrow therapy approach for human cases of Cooley's anemia.

Transplantation of "Spare Parts"

Techniques are available for getting relatively permanent homografting of tissues or parts of organ systems in the experimental animal. Ferrebee and Merrill [5] have reviewed these from the standpoint of possible surgical applications. Actively acquired tolerance results from the injection of mice or rats with adult homologous spleen or other cells at or near the time of birth. Animals so injected will later often permanently accept homologous skin grafts or ovarian grafts of the same type as that of the injected spleen. Isologous, homologous, and heterologous bone marrow transplantation after irradiation is itself an example of organ system transplantation. In the last two cases the successful transplants are established, with the complication of a relatively permanent and severe impairment of the host animal's immune status. When homologous fetal blood-forming cells are used, this complication may not be so severe. Once the foreign bone marrow is established, however, other kinds of donor tissue, such as skin, ovary, and tumors, can be accepted by the foreign host. Attempts to use these techniques in kidney homotransplantation in man were made by the group at the Peter Bent Brigham Hospital.[6]

The development of new methods of organ system transplantation, cell preservation, and cell separation will probably also result from continued efforts to extend the experimental applications of bone marrow transplantation research. Some of the ideas, such as replacement of the intestinal mucosa and repopulation of liver parenchymal cells, seem a little remote with our present techniques. Cell separation research is still new, and what might be accomplished with suitable living-cell preparations of various organ systems needs to be determined. For a different approach to the problem of "cellular coaptation" the reader is referred to the paper by Weiss and Andres.[7]

Treatment of Advanced Cancer

Under this heading we shall consider experiments where some form of whole-body irradiation or chemotherapy is used to slow down or eradicate tumors cells that are actively growing in an animal. The use of external x- or γ-irradiation, partial or whole-body, in relatively mild doses is a well-known technique in palliation of human leukemia. Whole-body irradiation with an internal emitter (P^{32}) is also well estab-

lished for use in human patients. Chemotherapy, another type of whole-body treatment of leukemia, is equally well established and constitutes our major research approach to the control of this disease.

With the advent of bone marrow transplantation and related techniques, contemplation of much higher total-body exposures, by either ionizing radiations or chemotherapy, seems feasible. The earliest experiments in trying to develop this idea were carried out in Lorenz' laboratory. It was found that 800 r of whole-body x-rays gave 32% to 92% permanent regression of a *localized* transplantable lymphoma in Strain A mice. The surgically exteriorized spleen * was lead-shielded during the irradiation. Forty-seven mice survived the irradiation procedure, and 29 had permanent regressions. Strain two guinea pigs, carrying a *generalized*, rapidly growing lymphatic leukemia, were exposed to a fractionated dose of whole-body x-rays totaling nearly 2,000 r. After the final irradiation, they were given isologous bone marrow intravenously. A significant remission of the leukemic process with a normal blood picture was present before the disease relapsed, and the animals died of leukemia. Several groups have repeated this type of experiment using either total-body irradiation or radical chemotherapy to kill tumor cells and bone marrow or spleen transplantation to treat the damage to normal hematopoietic tissues by the cancerolytic agent. A limited measure of success was reported in this work. Other studies were less encouraging. In patients with leukemia these techniques have given an occasional temporary remission.

We believe there is a growing opinion that the definitive control of an established disseminated cancer with techniques available now must be attempted when the smallest number of tumor cells are present. This means that the greatest effort to sterilize the residual tumor cells will be made after primary surgical excision of a tumor or during remission of leukemias. "Greatest effort" here refers to radical chemotherapy or radical radiotherapy. The physican probably will have to learn how to induce an extremely critical state in the patient, at the proper time, to sterilize residual tumor cells, and then to induce recovery of injured normal tissues. His major tools will be techniques to control critical systems injured or destroyed by the radical cancerolytic agents. This accounts for the interest in autologous bone marrow transplantation to control damage, to at least one organ system.

In experimental animals use of graded doses of the cancerolytic agent, followed by transplantation of the affected tissue as a bioassay, can give some idea of how many times the L. D. $_{50}$ dose of the agent is necessary to sterilize the affected tissue of its tumor cells.[8,9] Burchenal, for example, finds an x-ray dose of 3,500-12,000 r necessary to sterilize a series of transplantable leukemias (personal communication, 1959). Mechlorethamine (nitrogen mustards) required 5-10 times the L. D. $_{50}$ dose to sterilize, and 100 times the L. D. $_{50}$ of folic acid antagonists would not sterilize the transplantable tumor.[9] With dose values obtained in this way, we might be able to determine the chances of success in a tumor patient treated by radical cancer therapy when the fewest tumor cells are present. We think that the sterilizing dose values of any new cancer chemotherapeutic agent should be determined in the experimental animal and, if it has not already been done, of known agents as well. Comparison of these values among different agents or combinations of agents might be our best guide in directing our vast cancer chemotherapy research program. Furthermore, dose values should help us to decide how much effort to put into the adjunctive measures, such as autologous bone marrow transplantation or

* L. O. Jacobson and his associates at the Argonne National Laboratory were first to show that spleen shielding or spleen protection prevented death from whole-body irradiation in mice. They were also first to use intraperitoneal transplantation of infant mouse spleens or mouse embryos after whole-body exposure to produce recovery from the acute radiation syndrome.

gut transplantation, that will be needed to induce a satisfactory return from the severe toxic state. This is a difficult way to attack the definitive control of an established cancer. However, the statistical nature of killing curves for any kind of living material shows that the percentage advantage in dealing with the smallest possible population will hold for any conceivable therapeutic attack. Cancer therapy techniques that we imagine for the future, such as immunoselective, chemoselective, or virus control methods, would only allow less radical treatment.

Prevention of Induced Leukemia

Mice, rats, guinea pigs, and man develop leukemia of one type or another after sufficient radiation exposure. In irradiated mice a lymphosarcoma originating in the thymus is a usual type of tumor. Myeloid leukemia, possibly originating in the spleen, is induced by radiation in the RF strain of mice. The radiation-induced thymic lymphosarcoma can be prevented by isologous bone marrow injection at the proper interval after irradiation. Shielding blood-forming cells during irradiation also prevents thymic lymphoma. Kaplan and his associates at Stanford University have made many studies on this experiment. They find that bone marrow treatment causes the thymus to recover quickly from its extreme radiation necrosis. Other groups have shown that the irradiated thymus changes to the type of the bone marrow donor after homologous and heterologous marrow transplantation for whole-body irradiation.

It still is not settled whether the quick restitution of the thymus or the cellular repopulation is the mechanism responsible for preventing leukemogenesis.

Upton and Furth found that the incidence of radiation-induced myeloid leukemia in RF mice was reduced when bone marrow areas were shielded during irradiation. Many chronically γ-irradiated guinea pigs developed an acute lymphoblastic leukemia after the exposure was stopped. Although isologous bone marrow transplantation enabled these guinea pigs to recover from the subacute radiation syndrome, it did not prevent the development of leukemia. Many interesting and important problems are still unanswered in this area. Hormonal and chemical carcinogen-induced thymic leukemias in mice have not been studied from the standpoint of the bone marrow transplantation experiment. We do not know how other kinds of leukemias† might be influenced by bone marrow transplantation, since ready experimental production is limited to the types mentioned.

Considerably more experimental work must be done before we know how to make use of these animal studies in preventing induced leukemia in man.

Prevention of Spontaneous Leukemia

In some strains of mice, such as the AKR, nearly every animal dies of spontaneous thymic leukemia. Egon Lorenz, one of the pioneers in bone marrow transplantation, set up special genetic experiments in which he could give bone marrow from a nonleukemic strain of mice to a young compatible host that would be expected to get leukemia later in life. The preliminary studies indicated that the bone marrow injections reduced the incidence or rate of appearance of spontaneous leukemia.

As far as we know, experiments of this type have not been done in any other species.

Immunologic Applications

Many interesting new discoveries seem to be developing, particularly from the quantitative immunologic studies that were made possible in part through bone marrow transplantation in irradiated animals. Immune status determinations on mice that have survived from many months after exposure to lethal total-body irradiation and treatment with adult foreign bone marrow showed these animals to be immunologic

† It should be noted that in animal research nearly all tumors of reticular tissues are referred to as leukemias. We have usually used the term in this sense throughout the paper.

cripples and not really in a state of true tolerance, as many experimentalists assume. It was further shown that, even at very high radiation doses, damage to the immune mechanism is a relative matter, depending on the type of antigen injected.

Our studies on the lymphatic tissues of irradiated mice treated with foreign bone marrow or spleen showed this tissue to be the major site of pathologic changes in the foreign bone marrow or foreign spleen reactions. These tissue studies in irradiated animals fit into the general scheme of cellular reactions that make up the anatomic basis of the immune mechanism in intact animals. In fact, the histologic examinations suggest that it might be possible to set up morphologic criteria for including or excluding an immunologic component in diseases where this mechanism is one of several listed in the formal genesis. Rheumatoid arthritis, for example, might be studied in this way.

Also immunologically important, at least for tissue transplantation, is the chemical nature of the antigenic material that causes homo- and heterograft rejection. Preliminary studies by Makinodan and Billingham and their associates point to involvement of polysaccharide-like substances. Research in this critical area should eventually demonstrate a chemical basis for one of the important individuality differences. Since we think that tissue antigens are genetically determined, these polysaccharide substances could be thought of as the phenotypic expression of gene action.

The genetic theory of tissue transplantation has been used in transplantation of bone marrow and other blood-forming tissues. Owen[10] has reviewed the subject. For detailed studies, see the work of D. Uphoff, J. J. Trentin, E. E. Schwartz et al., L. J. Cole et al., P. L. T. Ilbery et al., D. W. van Bekkum and O. Vos, and others, cited in References 1 and 2.

Of special note is the work on radiation damage to the recognition factor in antibody formation. Radiation gives less damage to a mouse's ability to recognize sheep red blood cell antigens than to a comparably irradiated mouse's ability to recognize rat red blood cell antigens. Extending this finding to age studies, workers in this laboratory found that very young mice could recognize sheep red blood cell antigens earlier than they could recognize rat red blood cell antigens.

Some Basic Research Considerations

We usually think that the homeostatic mechanisms in mammalian species, as well as other forms, have been selected during evolution to give the cell an optimal environment for its activities, and also for recovery from an injury. The latter assumption is obviously too simple a concept. In supralethal whole-body irradiation, no bone-marrow stem cells can recover quickly enough to prevent death of the animal from hemorrhage and infection. Yet it is clear from irradiated, foreign bone-marrow-treated animals showing reversions to host type cells that, given sufficient (one or more months) time, the injured stem cells recover. How can this kind of recovery process be influenced? One area of research is called chemical protection. The presence of certain chemical substances at the time of irradiation reduces the effective dose of the radiation. Apparently, critical biochemical or biophysical steps in the injury process are interfered with. Patt,[11] Bacq,[12] and others have reviewed the literature in this field.

The most striking compound that we have seen in the protection of supralethally irradiated mice is AET (S,2-aminoethyl-isothiuronium bromide hydrobromide), discovered by Doherty and his associates in this laboratory. Doherty believes that AET's major function is to neutralize, by electron donation, free radicals formed by the radiation decomposition of water before they do biochemical damage. The AET-free radical formed in this process is not so aggressive since its unique structure permits a measure of resonance stabilization, giving it a much

longer half-life. It lasts long enough to react with another free radical and thus acts as a chain-terminating agent in preventing the free-radical-induced chain depolymerization of important biochemical molecules. This is also in agreement with its action in reducing damage by nitrogen mustards.

Doherty also thinks we could better understand the biochemical mechanism of radiation injury if we knew what critical biochemical processes were protected by the drug. Some toxicity studies have been made with AET in human beings, with a view toward its use for protection against radiation therapy or nitrogen mustards.

The other area of research that has to do with recovery from injury depends on an entirely different, usually genetic, approach to the problem (for a review of the literature see Hollaender and Kimball [13]). Processes that lead to chromosome aberration, mutation, or cell death in plants, bacteria, protozoa, and insects may be stopped or altered by various postirradiation procedures that change the metabolism of the cells in a favorable manner. It is important to note that the techniques must usually be carried out during a limited period of time after irradiation, when the biochemical events that result from the initial insult are taking place. The mere fact that radiation-induced genetic damage can be modified in any living material is of great theoretical and practical importance. The studies referred to show that genetic injury does not appear instantaneously but requires time to develop. Studies by Russell et al.[14] on mice exposed to chronic γ-irradiation showed a much lower mutation rate than those given an equal dose of acute x-radiation. These investigators suggest the possibility of reparable genetic damage to explain the results; i.e., the low intensity of radiation allows time for spontaneous repair not possible with acute exposure. Studies on plants, bacteria, protozoa, insects, and other forms are essential both for understanding the basic mechanisms of irradiation and for understanding the process of chemical protection and true recovery from injury. The ideas mentioned apply to most kinds of cell injury, not to radiation injury alone.[15,16]

Applications to Man

In several countries clinical research groups are actively trying to work out the application of bone marrow transplantation to problems of human disease. We have indicated most of these in the foregoing sections. Some of the work has been published, but much of it is still being evaluated and extended.

The most obvious application of bone marrow transplantation to man would be for victims of lethal total-body irradiation accidents. Three serious accidents occurred in 1958. In the Oak Ridge Y-12 Nuclear Excursion, the most heavily exposed persons were examined and determined not likely to have had lethal exposures.[17] The decision was made not to give bone marrow. No deaths occurred. A reactor accident in Yugoslavia resulted in serious exposures for six workers.[18] One victim was judged to be sublethally irradiated and was not given a bone marrow injection. The other five all received transplantation of blood-forming cells. One of the five died, and the others recovered from the acute radiation syndrome.[20]

An accident in Los Alamos resulted in the high supralethal exposure, with very quick death, of one worker and sublethal exposure in another.[19] No bone marrow transplantation was attempted.

We think it is most encouraging that there is now a fairly extensive research program on recovery from massive radiation injury in laboratory animals, whereas 10 years or more ago there was practically nothing that could be done for supralethal exposures. Many preliminary clinical trials to make use of the research data are being carried out.

New ways have been suggested to treat chemical damage to hematopoietic tissues, and new approaches to treatment of some kinds of hereditary disease are now possible.

Much new information on the tissue homotransplantation problem has developed from experiments with animals and human beings. Possibilities of transplanting organ systems by way of the blood stream or other special routes need now to be considered.

A possible role for bone marrow transplantation in the treatment of leukemia and some other cancers is suggested by the animal and human experiments. From the studies on the prevention of leukemia we might gain insight into the pathogenesis of this disease.

It is also clear that, by the time our present experimental applications have been worked through for application to man, new approaches to protection and recovery from radiation injury should be forthcoming from studies on micro-organisms and other material.

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Tetracycline Fluorescence in Malignant Tumors and Benign Ulcers

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The purpose of this report is to describe our studies on tetracycline-induced ultraviolet light fluorescence in patients with malignant tumors or nonspecific ulcerative skin lesions. In addition, experimental animals bearing skin ulcerations, intradermal abscesses, and subcutaneous non-neoplastic proliferative tissue reactions were studied. This study was undertaken following reports of ultraviolet fluorescence of malignant tumors subsequent to systemic tetracycline administration,[1,2] apparently due to localization and persistence of tetracyclines within areas of neoplastic disease.

Materials

The following groups were studied:

A. Groups receiving tetracyclines

1. Nine cases of carcinoma. Three cases of infiltrating squamous-cell carcinoma of cervix, all with eventual hysterectomy; one case of infiltrating squamous-cell carcinoma of skin of leg, due to depilatory x-ray treatment 14 years previously; one case of squamous-cell carcinoma of penis with extensive local infiltration; one case of metastatic testicular chorionepithelioma to skin of groin; one case of adenocarcinoma of pancreas with diffuse intra-abdominal metastases studied at autopsy 4 weeks after the last tetracycline administration; two cases of undifferentiated bronchogenic carcinoma with extensive mediastinal infiltrates studied at autopsy 8 and 14 weeks, respectively.

2. Five patients with chronic nonspecific skin ulcers.

3. Fifteen rats with hydrochloric acid-induced skin ulcers.

4. Five rats with subcutaneous absorbable gelatin sponge U. S. P. (Gelfoam) pledgets.

5. Five rats with intradermal Staphylococcus aureus and Escherichia coli abscesses.

All rats were adult males of the Wistar strain, with an average weight of 200 gm.

B. Control groups of similar patients and experimental rats without tetracycline administration. In addition, gross and microscopic fluorescence surveys of numerous routine surgical and autopsy tissues were undertaken.

Methods

1. Gross fluorescence. All material was examined with a Mineralite ultraviolet lamp (3,600 A); attention was given to surface and cut-surface fluorescence.

2. Histologic examination of all material in routine hematoxylin-eosin sections and Papanicolaou smears were made to confirm the diagnosis.

3. Ultraviolet light fluorescence microscopy techniques and apparatus were those described by Vassar and Culling.[3]

4. Ultraviolet dark-ground illumination, with B.G. 12 or U.G. 5 excitor filter, but without an eyepiece barrier filter, was utilized for analysis and evaluation of autofluorescence.

5. Cytologic smears were taken and (*a*) air-dried, (*b*) fixed in alcohol-ether, (*c*) fixed in formol-alcohol, (*d*) fresh frozen in isopentane, cooled to —140 C, and stored in an airtight container in Deep Freeze for prolonged storage without apparent deterioration (at present up to 10 months).

6. Sections were cut at 3μ in a cryostat at —15 C, utilizing tissue that had been fresh frozen in isopentane at —140 C.

7. Skin ulcers were induced on the back of the rat by the following technique[4]: Air, 20 ml., is injected subcutaneously, to produce an air sac, followed by the injection of 1 ml. 0.1 N hydrochloric acid. An ulcer is produced within 12 to 36 hours with sterile pus formation deep to a large, scabbed area. Tetracycline was administered the same day that the ulcer developed.

8. Pledget technique used was that described by Taylor and Saunders.[5] Tetracycline was administered 48 hours after pledget implantation.

9. Administration of tetracyclines to patients was in the form of tetracycline (Cosa-Tetracyn*), 500

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mg., orally, twice daily, for two days. Experimental animals received oral tetracycline (in solution, using a stomach tube) as a single dose, corresponding to 100 mg. per kilogram of body weight.

In all experiments in Group A, fluorescence studies were performed before and after tetracycline administration when possible; biopsy material was also taken in conjunction with the routine cytologic smears.

In order to ensure complete and accurate histologic identification of fluorescent and nonfluorescent material, the following technique was devised: An immobilized field was visualized with ultraviolet light and a specific locus identified with a pointer-eyepiece system: Following this, a drop of toluidine blue was placed over the field and the light source altered to bright light. Serial microphotography * was also performed in several instances.

The fluorescent color of tetracycline in tissues and smears was visually compared with that of the pure powder and its solution in distilled water.

Results

Smears preserved by the techniques described revealed complete quenching of fluorescence by formol-alcohol but only slight quenching by alcohol-ether, with some shrinkage of cells. Air-dried and frozen smears gave excellent results.

Tetracycline Fluorescence.—Tetracyclines have a characteristic fluorescence, best described as a bright yellow color. The color in tissues and smears was identical with that of the pure powder and its solution.

Autofluorescence.—Gross and microscopic ultraviolet light autofluorescence may be a troublesome phenomenon, particularly the pale-yellow gross fluorescence of adipose tissue; this may be seen in fat close to tumor areas following tetracycline administration. Microscopic autofluorescence of lipofuscin (brilliant yellow-orange) and porphyrins (red) may be confusing to the inexperienced observer, but none has the characteristic color and distribution of tetracycline-induced fluorescence. Occasional difficulty may be experienced in the microscopic interpretation of yellow fluorescent histiocytes, which contain cytoplasmic or surface-absorbed granules of lipoprotein (?) material; their identification must be verified without use of a yellow barrier eyepiece filter.

GROUP A (Tetracycline-Treated)

1. *Patients with Carcinoma.*—In every case there was typical fluorescence in both gross and microscopic preparations. The gross fluorescence was invariably confined to the tumor area and often confined to a narrow band lying just inside the advancing edge; the intensity of fluorescence varied considerably from patient to patient.

Two patients with carcinoma of skin developed minimal gross surface fluorescence 24 hours after administration of the drug, and was most intense after an interval of 48-72 hours. The fluorescence then diminished slightly for 24-48 hours, following which it showed no appreciable change for 21 days. In the autopsy cases, brilliant specific fluorescence was present up to 14 weeks after the last recorded administration of tetracyclines. Other drugs prescribed for these patients included mercurial diuretics, digitalis, tranquilizers, and penicillin; these were all tested both in the crystalline form and in solution, and none showed even a minimal fluorescence. Smears and cryostat-cut serial sections showed fluorescence was invariably confined to macrophages and tissue debris in the tumor stroma. The macrophages exhibited yellow granular cytoplasmic fluorescence. In no case was a malignant cell seen to fluoresce.

2. *Patients with Nonspecific Skin Ulcers.* Of five patients examined daily for 21 days, two showed a localization and brilliance of fluorescence for 48-72 hours after treatment; the fluorescence then diminished rapidly until none could be seen, except in scab formation, where it persisted. Three others developed a less intense fluorescence, which faded rapidly 24 hours after administration of the drug was stopped. Microscopic fluorescence was confined during this period to macrophages and tissue debris.

3. *Hydrochloric Acid-Induced Skin Ulcers.*—These ulcers showed minimal gross

* We wish to acknowledge our gratitude to Pfizer Company, Montreal, for supplies of Cosa-Tetracyn and generous donation of microphotography equipment.

fluorescence, commencing 24 hours after administration of the drug, which faded rapidly except in scab formation. This fluorescence was almost invariably confined to the immediate surface area. Typical fluorescent macrophages were detectable microscopically.

4. *Rats with Subcutaneous Absorbable Gelatin Sponge Pledgets.*—Minimal fluorescence was confined to scab formation in the incision site, with no demonstrable gross or microscopic fluorescence in the granulation tissue surrounding the pledget.

5. *Rats with Intradermal Injections of S. Aureus and E. Coli Communis.*—Fluorescence was confined to the small scab areas, possibly due to the fact that administration of the tetracycline caused rapid regression of the abscess with healing. An interesting feature of all animal experiments was the persistent specific fluorescence of bones, particularly in the ends of long bones. This was observed over periods up to three weeks.

GROUP B (Control Groups, No Tetracyclines)

In no instance was characteristic gross or microscopic fluorescence detected. Autofluorescence was present, as described above.

Comment

These findings confirm the presence of localization and persistence of tetracycline-induced fluorescence in a number of human malignant tumors and their metastases. In addition, however, to persistent localization of tetracycline fluorescence in bone,[6] there appears to be some temporary persistence of fluorescence in nonspecific ulcerative lesions, particularly if scab formation is present. These findings are supported by the recent work of Häkkinen and Hartiala[7] on tetracycline fluorescence in experimental ulcers; these authors, too, found the fluorescence limited to the ulcer margins with apparent concentration and localization in regions of necrotic debris rather than areas of regenerative tissue proliferation. Their explanation of the mechanism of tetracycline fixation by the change in degree of polymerization of ground substance is not supported, however, by our failure to demonstrate fluorescence in areas surrounding absorbable gelatin sponge pledget implants where similar changes in ground substance polymerization occur.

Of considerable interest is our finding that tetracycline fluorescence is confined invariably to histiocytes and debris within the tissue stroma in malignant tissues; malignant cells invariably failed to exhibit fluorescence. These findings are in accord with the work of Ludford[8] and Duran-Reynals[9] on the localization of various colored materials in tumor areas following blood stream injection of cancer-bearing experimental animals. They found similar localization and persistence within tumor stroma and mesenchymal cells, rather than neoplastic cells. The possibility that materials may localize and persist in neoplastic stroma as a result of tissue destruction, repair, and marked histiocytic activity is perhaps suggested by our findings that some tetracycline fluorescence may be detected in nonspecific skin ulcerations. However, some more specific mechanism must be operative in order to explain the persistent localization in malignant tumor tissue, and yet its absence in both the tissue reaction provoked by pledgets and the exudates beneath the scab formation in the experimental ulcers. The mechanism of tetracycline localization is unknown; we may speculate, however, that the sequence of events may be initiated by transudation or exudation of free and protein-bound[10] tetracycline into areas of tissue activity. Owing to some unknown local biological conditions, such as polypeptide complex linking[11] or calcium metabolism,[6] there are a local binding and precipitation of tetracycline in the area. It should be noted, finally, that there is experimental evidence[12] that tetracyclines may show temporary localization within the reticuloendothelial systems of the body.

Summary

Tetracycline-induced ultraviolet light fluorescence of human malignant tumors was confirmed and found to be confined to histiocytes and debris within the mesenchymal stroma only; malignant cells were found to be nonfluorescent. In addition to persistent fluorescence in bone, some temporary localization and persistence were detected in nonspecific skin ulcerations.

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Addendum

Since completion of this manuscript an important contribution to this subject has appeared in which it was found that precipitation of lipoprotein-tetracycline complex will occur in the presence of calcium ions.[13]

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Histochemical Study of the Adult Aorta

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The presence of mucopolysaccharides in the human aorta has been recognized for many years. Schultz,[1] Ssolowjew,[2] and Wolkoff[3] noted the regular occurrence of these substances and indicated that the quantity increased with age. They attributed this increment to concomitant damage of elastic tissue associated with arteriosclerosis. The property of metachromasia and the relation of the polysaccharide to cartilage and amyloid were likewise noted by Schultz.[1] He suggested that the staining feature indicated the presence of chondroitinsulfate. Erdheim[4,5] regarded the increasing amount of polysaccharide to be abnormal, and possibly a contributing factor in dissecting aneurysm; Schultz,[1] however, considered it to be a nutritive substance. Meyer[6,7] demonstrated two types of aortic acid mucopolysaccharide (chondroitinsulfates B and C) by chemical extraction procedures. They could be distinguished on the basis of lability (Type C) to testicular hyaluronidase digestion. Subsequently, Deiss and Leon[8] demonstrated hyaluronic acid derivatives in cardiac valvular tissue.

In pathologic conditions the accumulation of acid mucopolysaccharide in the aorta has been correlated with aging and arteriosclerosis by Bunting and Bunting,[9] by Rinehart and Greenberg,[10] and by Taylor.[11] All but the first indicated an association with damage to the elastica. Bunting and Bunting, on the other hand, described a gradual increase in quantity up to age 40, followed by a progressive reduction. Accumulation of mucoid material in dissecting aneurysm was described by Erdheim,[4,5] by Cellina,[12] and by Gore.[13] The current increased interest in the Marfan syndrome[14] has directed attention to the association between degeneration of elastica and an apparent increase in basophilic mucoid substance in the aorta. Experimental substantiation appeared with the demonstration of similar alterations in animals given sweet pea extracts or proprionitriles.[15-17]

The present study was undertaken to investigate the pathogenesis of dissecting aneurysm. Previous investigations of this type have failed to evaluate abnormalities with formal controls. Accordingly, a large series of aortas were obtained at random and subjected to both histologic and histochemical study.

Materials and Methods

Sections of the ascending aorta were obtained at necropsy from 200 adults (over 16 years of age). The ascending segment was selected because this area represents the most frequent point of origin of dissecting aneurysm.[18,19] The sections thus obtained were fixed in 10% buffered formalin and embedded, using the Swiss-roll technique, thus providing a section representative of the entire ascending aorta. Where necessary, decalcification was accomplished with 10% edathamil (Versene).

Paraffin-embedded sections were examined by a variety of histochemical techniques and special staining procedures (Table 1). The amount of acid mucopolysaccharide present was graded (0 to 4+) on the basis of the degree of blue coloration with the Alcian blue stain and the amount of metachromasia with toluidine blue.[20,21] Other abnormalities, such as severity of arteriosclerosis, mural fibrosis, and destruction of elastica and of smooth muscle, were also tabulated. In this fashion, data were gathered concerning the variation in com-

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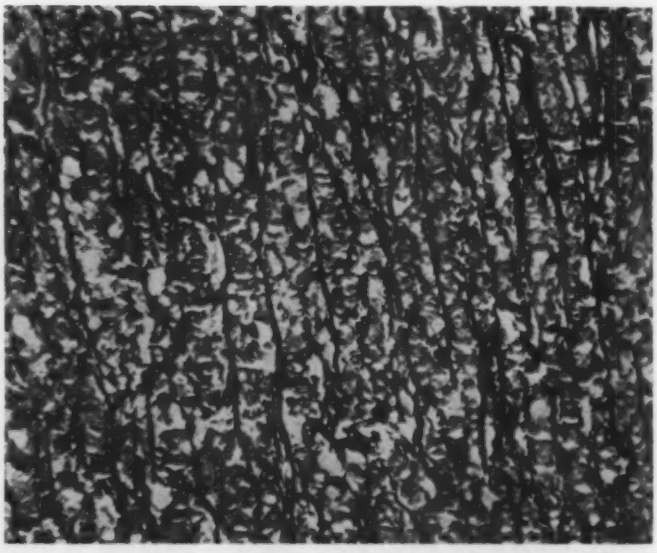


Fig. 1.—Appearance of the central elastica of an essentially normal aorta. Black lines represent elastic plates paralleling long axis of aorta. Note variability in thickness of fibrils and appearance of discontinuity of many plates in this routine section. Verhoeff stain; ×450.

position of the wall of the aorta under a range of circumstances.

Results

The components of the wall of the aorta as outlined by histologic and histochemical procedures are as follows:

1. *Elastic Tissue.*—Elastic plates made up approximately 20% of the volume of the fixed, embedded aorta. The elastic structure of the aorta was readily demonstrable with special techniques, such as the Verhoeff stain or the Gomori technique.[20] It could also be demonstrated without staining by phase microscopy of unstained sections. The elastica was arranged as parallel, wavy, refractile layers averaging 2μ to 4μ in thickness (Fig. 1). (This curvature is believed to represent an artifact of the shrinkage and contraction in sectioned plates.) In the ascending aorta the count ranged between

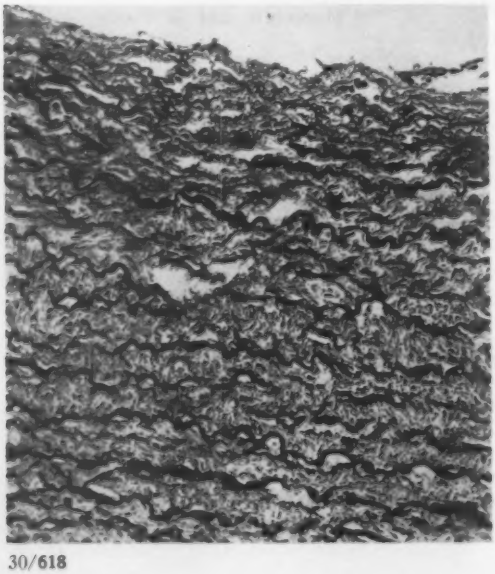


Fig. 2.—The intimal portion of a "normal" aorta (above). Elastica here is similar in character to that seen in Figure 1. Note lack of any discrete internal elastic lamella. Even in this section there is already fragmentation of innermost layers of elastica and increase in connective tissue. Verhoeff stain; ×160.

68 and 122 plates. The arrangement was quite regular in sections parallel with the long axis of the aorta. However, if the section was cut tangentially, the appearance of irregular interdigitation and reticulation was encountered. Paraffin sections revealed apparent breaks in many lamellae, although a few could be followed throughout their entire length. Variability in size and arrangement of fibrils were characteristic, many appearing finer than the major parallel elastic laminae. These observations are undoubtedly the consequence of the morphology of the aorta as seen in three-dimensional reconstructions.[22] The fenestrated plates contain both fine and coarse elastic fibers. The presence of numerous foramina presumably accounts for the apparent interruptions of the elastica noted in routine sections. There was no discernible internal elastic lamella in the normal aorta (Fig. 2). On the other hand, in atherosclerosis one or more thickened and irregular plates could occasionally be seen separating the intima from the wall of the aorta.

Aging did not appear to influence the structure of the elastica except insofar as the increasing incidence of severe atherosclerosis with age caused damage to the media. Table 2 summarizes the age incidence of the 14 examples of severe damage to the elastica. These cases were all considered to have maximum (4+) atherosclerosis (see below). Milder degrees of arteriosclerosis were not accompanied by significant damage to the media, although superficial fragmentation of elastic plates was common in immediate proximity to the

TABLE 1.—*Staining and Histochemical Studies of Ascending Aorta* *

Structure	Elastica	Smooth Muscle	Acid Mucopolysaccharide	Collagen	Reticulin
H. & E.	Refractile	Fibrillar eosinophilic	Blue	Eosinophilic fibrillar	
Verhoeff (elastic)	Black	0	0	0	0
Van Gieson	Unstained	Yellow	Yellow	Red	Pink
Aniline blue (Mallory)	0	0	0	+	+
PAS	0	Usually +	0	Weak +	+
Diastase-PAS	--	Usually 0	--	+	+
Acetylation-PAS	--	0	--	0	0
Bisulfite-PAS (2 hr.)	--	Usually +	--	0	0
Direct Schiff	0	0	0	0	0
Alcian blue	0	0	+	0	0
Alcian blue-PAS	0	Usually red	Blue	Pink	Red
Protein reactions	+	+	0	+	+
Alcian blue-protein	Red	Red	Blue	Red	Red
Toluidine blue, pH 2	0	0	<5% metachromatic	0	0
Toluidine blue, pH 4	0	0	All metachromatic	0	0
Toluidine blue, pH 6	0	0	All metachromatic	0	0
Lability to testicular hyaluronidase †	--	--	90% labile	--	--
Stability to streptococcal hyaluronidase ‡	--	--	Stable	--	--
Sulfation, toluidine blue, pH 2	--	--	Increases metachromasia	--	--
Methylation toluidine blue	--	--	Reverses metachromasia	--	--
Saponification Methylation-toluidine blue	--	--	Fails to restore metachromasia	--	--
PTAH	Blue-purple	Blue	Blue	Red	Red
Feulgen	0	Nuclei +	0	0	0
Lability to ribonuclease digestion	--	--	Stable	--	--

* Symbols: plus sign (+)=positive; 0, negative; ..., not ascertainable, or not observed.
† Nutritional Biochemical Co., Cleveland.
‡ Courtesy of Wyeth & Company, Philadelphia.

TABLE 2.—*Ages of Patients with Severe Medial Damage*

Age Groups	Number in Series	Number with Medical Damage
10-19	3	0
20-29	5	0
30-39	16	1 *
40-49	19	2
50-59	39	3
60-69	49	3
70-79	45	4
80-89	21	1
90-99	3	0
Totals	200	14

* Syphilitic aortitis.

intima. However, in severer grades of arteriosclerosis (3+ and 4+), the elastica was affected to a considerable degree. With 3+ atherosclerosis, alteration of the elastica beneath the plaque occasionally extended through as much as one-third of the media. With 4+ lesions, destruction of the elastica throughout virtually the entire media beneath plaques was not uncommon (Fig. 3). Occasionally beneath fibrous plaques with little or no protrusion in the direction of the lumen, the elastica manifested extensive damage with fibrosis and vascularization (Fig. 4). In a single case of syphilis, destruction of the elastica was extensive and was presumed to be related to the inflammatory process, rather than to the atherosclerosis accompanying the lesion (Fig. 5). There was one instance, in a 65-year-old patient, of focal degeneration of the elastica of mild degree, apparently unrelated to arteriosclerosis or inflammation. The lesions were mild and not of the order described in Marfan's syndrome [14] or experimental lathyrism.[15-18]

There were two cases of senile ectasia (dilatation of the ascending aorta associated with senility) without arteriosclerosis. In these, the elastica showed no significant histologic abnormalities. There were two instances of dissecting aneurysm, both of localized nature and involving different segments of the aorta from that examined (descending thoracic and abdominal). In one of these cases severe atherosclerosis had caused medial damage, and there was considerable mucopolysaccharide in the ascending aorta (3+). In the other, both alterations were graded as 1+.

2. *"Ground Substance."*—Acid mucopolysaccharide was always present, forming acellular pools in the interstitial tissue between elastic lamellae. Even in undamaged aortas there was considerable variation in quantity. However, when sections were cut tangentially, the apparent width of the

Fig. 3.—An arteriosclerotic plaque is causing marked destruction of the inner half of the elastica. There is almost total absence of elastic plates in the involved area. Replacement is by a mixture of connective tissue and loosely organized extracellular acid mucopolysaccharide. Verhoeff stain; ×25.

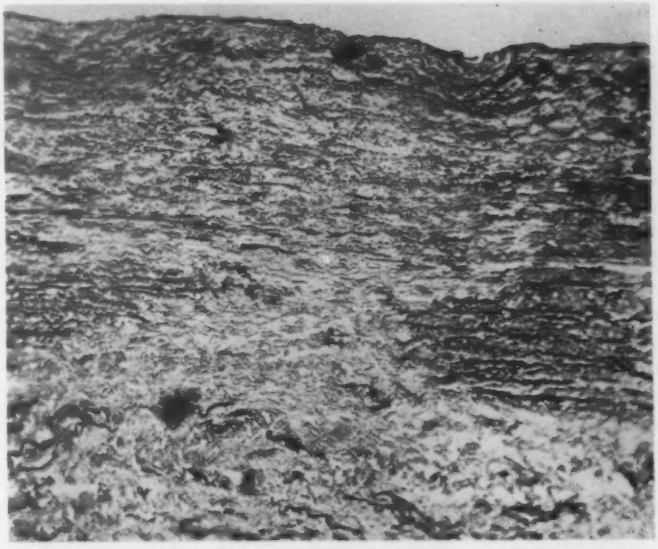


Fig. 4.—A fibrous plague, protruding only minimally above the intimal surface, is associated with extensive destruction of the underlying media and fibrous replacement. Verhoeff stain; reduced to 86% of mag. ×60.

spaces between elastic plates was greater, and the polysaccharide content appeared more prominent, although presumably it was not actually increased. This component was characteristically weakly basophilic in sections stained with hematoxylin and eosin (Fig. C1). It was metachromatic with toluidine blue (Fig. C2), a characteristic

Fig. 5.—Aortitis, resulting from syphilis, has caused marked disruption of the elastica of the aorta. Replacement is by connective tissue and extracellular mucopolysaccharide. Associated arteriosclerosis has caused damage to the intima. Verhoeff stain; ×25.

that correlated well with areas manifesting positivity with Alcian blue (Fig. C3). It did not stain with the periodic acid-Schiff (PAS) method or the coupled tetrazonium reaction. On the basis of a standardized technique of staining with toluidine blue,[21] a quantitative estimate of the amount of acid mucopolysaccharide present was made, using the 1+ to 4+ grading system. As previously mentioned, there was considerable quantitative variation in essentially normal aortas (Figs. C2, C4), and there was a tendency for somewhat greater quantities to be present in the portions closest to the intima (Fig. C2). The latter variation largely reflected the frequency of damage to the inner segment from atherosclerosis. Indeed, there was a tendency for the substance to accumulate in areas where damage was present, whatever the cause (see below).

The metachromatic substance was found to be labile to testicular (Fig. C5) but not to streptococcal (Fig. C6) hyaluronidase. The property of metachromasia was eliminated by methylation,[20] but was not restored by subsequent saponification with KOH.[23-26] Metachromasia was maximal in the range of pH 4 to pH 6 and virtually absent at pH 2. Sulfation[27] caused an increase in the amount of metachromasia at pH 2. There was no staining with aniline blue and a yellow coloration with the Van Gieson stain. The source of the mucopolysaccharide could not be ascertained. Mast cells were rarely noted in sections stained with toluidine blue and seemed an unlikely precursor. The wall of the aorta contained spindle cells in proximity to fibers identifiable with the Van Gieson stain as collagen;

Fig. C1.—Basophilic material in spaces between elastic fibrils in aorta from case graded at 3+. Hematoxylin-eosin stain; × 300.

Fig. C2.—A similar case (graded 3+) demonstrates metachromatic material in the same location. Intimal surface is to left. Toluidine blue stain; × 300.

Fig. C3.—The same case as in Figure C2 illustrates that material staining positively with Alcian blue occupies the same general area as the metachromatic substance. Red-staining material represents reticulum and smooth muscle fibrils. Alcian blue-PAS stain; × 300.

Fig. C4.—This section illustrates the degree of metachromasia encountered in a typical section of aorta graded at 1+. Comparison with Figure C2 indicates the marked variation encountered in the media in the absence of significant damage to elastica. Toluidine blue stain; × 300.

Fig. C5.—The same section as that illustrated in Figure C2 is shown after digestion with testicular hyaluronidase. There is marked diminution in metachromatic staining material. Toluidine blue stain; × 300.

Fig. C6.—The same section as that illustrated in Figures C2 and C5 is shown after digestion with streptococcal hyaluronidase. Metachromatic staining material is quantitatively similar to that in Figure C2 but considerably in excess of that in Figure C5. Toluidine blue stain; × 300.

Fig. C7.—An arteriosclerotic plaque (above) containing considerable metachromatic material. Toluidine blue stain; × 15.

Fig. C8.—This section illustrates a large arteriosclerotic plaque (above) with extensive calcification and fibrosis, but containing only minimal metachromatic material. Toluidine blue stain; × 15.

Fig. C9.—The media from a case of syphilitic aortitis is illustrated. Enormous pools of metachromatic material accompanied by loose connective tissue, fill spaces previously occupied by elastic fibers. Toluidine blue stain; × 300.

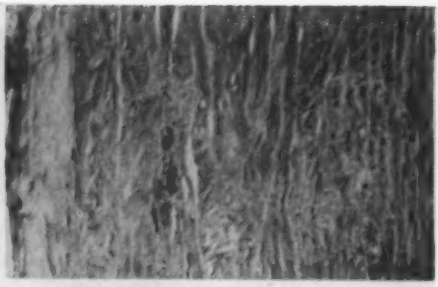
Fig. C10.—The same acellular focus depicted in Figure 7 is shown stained with toluidine blue. Note compression of elastic fibrils and marked diminution of metachromatic material in this area. × 200.

TABLE 3.—*Correlation of Age with Quantity of Acid Mucopolysaccharide*

Age	Total	0 to ±	1+	2+	3+	4+
10-19	3	0	1	1	1	0
20-29	5	0	1	2	2	0
30-39	16	0	5	6	4	1*
40-49	19	0	7	6	6	0
50-59	39	0	19	9	11	0
60-69	49	0	20	12	17	0
70-79	45	0	17	19	9	0
80-89	21	0	8	12	1	0
90-99	3	0	0	2	1	0
Totals	200	0	78	69	52	1

* Syphilitic aortitis.

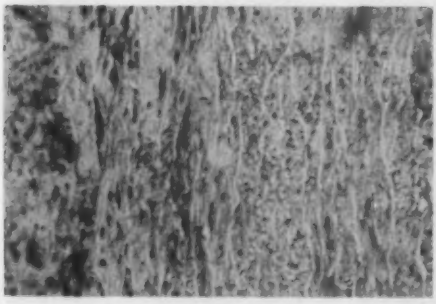
C1



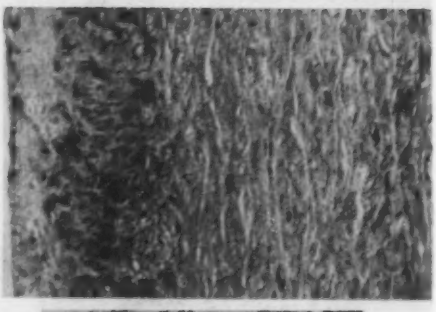
C2

C3

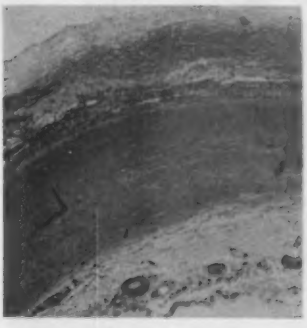
C4



C5



C6



C7

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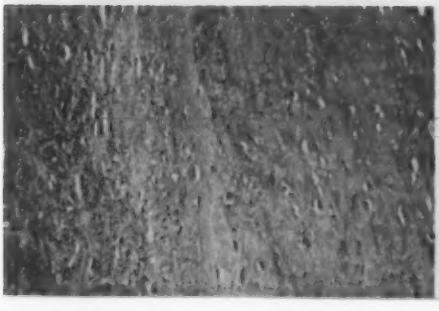
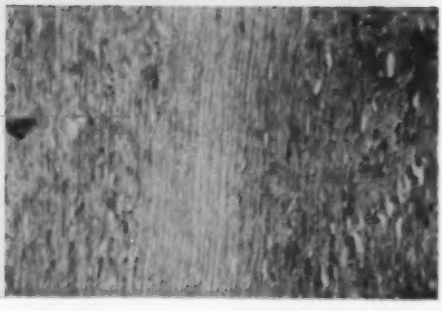


C9



C10

these fibrocytes could conceivably serve as the source for the mucoid material as well.

Table 3 summarizes the relationship of age to the quantity of acid mucopolysaccharide. There appears to be no significant variation with age, nor was there any difference between the age group above and below 40 years. There was, however, a direct correlation between arteriosclerotic plaques and the accumulation and localization of metachromatic material. In general, younger plaques revealed a large quantity of this substance (Fig. C7), while older lesions tended to be more fibrotic with less mucopolysaccharide (Fig. C8). The amount of mucoid substance tended to be greater in proximity to the intima, where damage due to atherosclerosis was severer (Fig. C2). When the media was damaged extensively, accumulations of metachromatic material were noted (Fig. C9). One case of syphilis with extensive medial destruction manifested the maximum accumulation of acid mucopolysaccharide observed.

Within the media, linear foci of loss of metachromatic substance were observed (Fig. C10). These corresponded to acellular foci with condensed, but intact, elastic lamellae, representing areas where smooth muscle cells and fibrocytes had disappeared. These areas stained pink with the Van Gieson stain, indicating the existence of residual collagen following the removal of mucoid substance.

3. *Smooth Muscle.*—The quantity of smooth muscle varied considerably but generally constituted 5% (or less) of the volume of the aorta. These spindle-shaped cells possessed processes that stained yellow with the Van Gieson stain and failed to stain with aniline blue techniques. Their arrangement, as well as their number, was rather haphazard, with some cells appearing parallel and others arranged at varying angles to the elastic plates. Counts of their numbers ranged from 50 to 100 per high-power field, and it was not uncommon to find small foci where no smooth muscle was apparent. Content of glycogen was characteristically limited to these elements; this polysaccharide was identified by PAS positivity, lability to diastase digestion, and resistance of

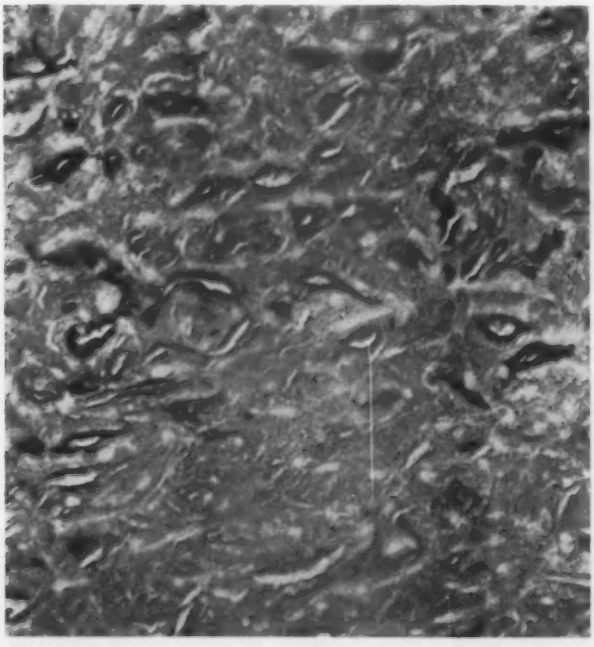


Fig. 6.—High-power view illustrates positive PAS reaction in smooth muscle cells in sections of media. Bisulfite-PAS stain; × 900.

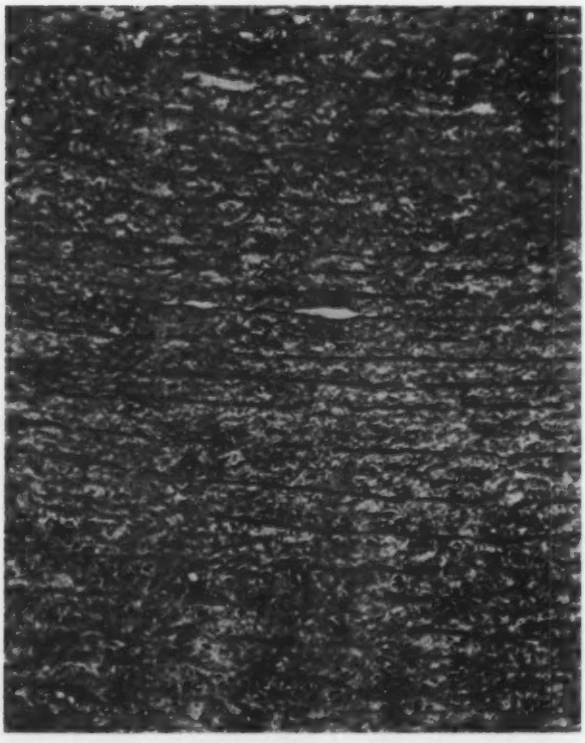


Fig. 7.—A linear acellular focus is depicted, occupying the central portion of the aorta. Although elastica and interstitial connective tissue persist, cells are totally absent. Hematoxylin-eosin stain; × 160.

the PAS positivity to blockade by prior bisulfite treatment for two hours [20] (Fig. 6). Other PAS-positive components (reticulin and collagen) were resistant to diastase digestion, and the PAS reaction was blocked by bisulfite. Acetylation, however, reversed the positivity of all PAS-staining components. Smooth muscle fibrils containing glycogen were orthochromatic with toluidine blue and probably metachromatic following sulfation, although the latter property was evaluated with difficulty.

As previously indicated, focal loss of smooth muscle was encountered in 15 instances. These lesions were characteristically linear and located within the media. Complete acellularity, loss of acid mucopolysaccharide, and compression of the otherwise intact elastica were characteristic in these areas (Figs. 7 and C10). Presumably, fibrocytes were lacking here as well. Although unassociated with atherosclerosis, there were commonly loss of cells and fibrosis in areas contiguous with atheromatous plaques extending into the media. In virtually all of the severer instances of atherosclerosis these lesions were prominent, but they were considered to be of different nature from the acellular foci within the media. The cause of the latter was unapparent. In five instances the lesions were of consider-

TABLE 4.—*Ages of Patients with Acellular Foci in Media*

Age Groups	Number in Series	Number with Acellular Foci
10-19	3	0
20-29	5	1
30-39	16	0
40-49	19	0
50-59	30	2
60-69	49	6
70-79	45	5
80-89	21	1
90-99	3	0
Totals	200	15

able extent, measuring up to 1 cm. or more in length; in the remainder they were focal and measured only 1 to 2 mm. Although most of these lesions occurred in older persons, one appeared in a 29-year-old patient (Table 4). The five more marked examples of acellularity all were in patients in the seventh and eighth decades of life. Since similar lesions have previously been related to hypertension, an attempt was made to ascertain the incidence of hypertensive cardiovascular disease in the cases manifesting this lesion and in the entire group studied. Of the 200 patients, 154 had adequate clinical and pathologic data for interpretation. Of these 64 (41.6%) had both significant elevations in blood pressure and pathologic evidence of hypertension (cardiomegaly of hypertensive type and arteriosclerosis). The remainder had neither. (Instances in which the clinical and pathologic data did not correlate or in which either data were inadequate were excluded from the tabulation.) Of the 15 patients manifesting acellular medial foci, 10 were considered to have adequate data for evaluation of the presence of hypertension. Four (40%) of these had hypertension. Three of the five cases with larger lesions were hypertensive while two were not.

4. *Connective Tissue.*—Collagenous connective tissue comprised 10% to 30% of the aortic wall. The fibrillar substances, collagen and reticulin, combined with the acid mucopolysaccharide, filled the spaces between elastic plates and smooth muscle. Both were stained with aniline blue techniques.[20] With the Van Gieson stain, the reticulin fibrils were weakly colored, while collagen staining was strong. Small fibrils were strongly PAS positive; larger bands stained a fainter pink. The PAS reaction in both was resistant to diastase digestion but labile to blockade by means of acetylation or pretreatment with bisulfite. Protein reactions were positive, and the component was orthochromatic with toluidine blue. The combination of Alcian blue and PAS staining and the coupled tetrazonium reaction (for proteins) clearly served to distinguish this component from the acid mucopolysaccharide; the latter reacted positively with Alcian blue but was negative with both protein reactions and the PAS stain.

Most of the variability in the quantity of the connective tissue component was related to atherosclerosis (see below). Within plaques themselves and in portions of media damaged by the extension of the atheromatous process, early there was an accumulation of metachromatic substance. Later, however, there was increase in collagen. Many of the sclerotic plaques were of the fibrous variety alluded to previously and subsequently. In the single case of syphilis much of the elastica was destroyed, and there was considerable replacement fibrosis.

5. *Intima.*—In the regions recognizable as normal, the intimal endothelium rested upon a thin areolar layer superimposed on the most internal plate of elastic tissue (Fig. 2). However, in most instances this layer manifested the maximal variation in composition because of the invariable occurrence of arteriosclerosis in the aortas examined. Most of the characteristics of the components comprising the plaque have been previously discussed (except lipids, which were not investigated). Some of the features of the process itself may be indicated here.

Arteriosclerosis was graded on a scale of 1+ to 4+. Those lesions graded 4+ manifested extension of plaques to involve the media deeply with extensive destruction of elastica (Fig. 3). Those graded 3+ showed more superficial extension of plaques into the elastica. When plaques were extensive, but significant medial involvement was absent or focal, a grade of 2+ was assigned. Instances of mild arteriosclerosis were graded 1+. Table 5 summarizes the relationship of age to degree of arteriosclerosis. In general, the incidence of severer atherosclerosis increased until the eighth decade and was reduced thereafter. This reduction undoubtedly reflected the fact that persons

TABLE 5.—*Correlation of Age with Arteriosclerosis*

Age	Total	0 to ±	1+	2+	3+	4+	3+ & 4+, %
10-19	3	0	3	0	0	0	0
20-29	5	0	5	0	0	0	0
30-39	16	0	7	6	2	1*	13.3
40-49	19	0	10	5	2	2	15.8
50-59	39	2	13	17	4	3	18.0
60-69	49	0	12	28	6	3	18.4
70-79	45	2	11	17	11	4	33.3
80-89	21	0	4	13	3	1	19.1
90-99	3	0	1	2	0	0	0
Totals	200	4	66	88	28	14	21.0

* Syphilitic aortitis.

with more severe atherosclerosis succumbed before attaining this advanced age.

Ulceration of atheromatous plaques occurred in 18 instances and was commonest in the group graded 4+. In four of these the ulceration extended into the wall of the aorta, thus forming short dissection tracts (Fig. 8). Accumulation of acid mucopolysaccharide was commonplace in the plaques and in the areas of media damaged by the plaques. An important variant of arteriosclerosis, the fibrous plaque, was characterized by minimal protrusion into the lumen and a tendency to cause destruction of the elastica and medial replacement fibrosis (Fig. 4). Foam-cell reaction and deposition of lipid were minimal. Presumably, this variety of arteriosclerosis was largely responsible in those instances in which severe arteriosclerosis was not recognized grossly or the lesion was classified as minimal or moderate in severity. Of 14 aortas graded 4+ arteriosclerosis microscopically, the lesion had been grossly classified as slight in 1, moderate in 7, and marked in 6. Of the 28 microscopically graded at 3+, the opinion had been minimal in 9, moderate in 13, and markedly sclerotic in 6. An additional observation presumably related to arteriosclerosis was the presence of major degrees of medial calcification in aortas with relatively slight atherosclerosis. The number of such instances encountered was 11, but this may not represent an accurate reflection of the actual incidence, since many of the most markedly calcified aortas were initially subjected to decalcification. Many showed a finely stippled medial calcification, and it

Fig. 8.—A large, partially fibrotic arteriosclerotic plaque (above) has become ulcerated, with the formation of a short dissection tract. Hematoxylin-eosin stain; reduced to 86% of mag. × 70.

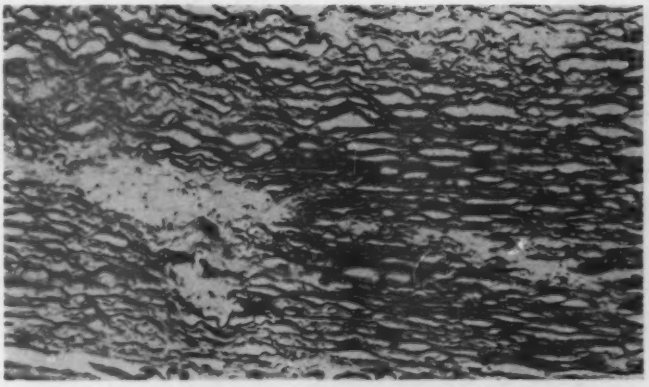


Fig. 9.—At the junction of the outer and middle third of the aortic media, vasa vasorum ramify parallel with the long axis of the aorta resulting in repetitive defects in the elastica. Verhoeff stain; reduced to 80% of mag. × 160.

was considered possible that this lesion might not be detected after decalcification.

6. *Adventitia.*—No external elastic lamella delineated the adventitial coat from the media. In the periphery of the wall, a few irregular elastic plates intermixed with collagenous fibers and acid mucopolysaccharide constituted the adventitia. A significant anatomic feature of the structure of the wall of the aorta was the distribution of the vasa vasorum. This pattern is clearly manifest in normal aortas but is accentuated when damage to the media induces extensive vascularization. It is thrown into prominence in sections stained to demonstrate elastic and fibrous tissues. The vessels perforate the adventitia and media perpendicularly to the longitudinal axis of the aorta and turn sharply at an angle of 90 degrees to ramify in a plane parallel to the long axis. They thus form a plexus of small thin-walled vessels, most commonly at the junction of the middle and outer thirds of the wall (Fig. 9). Thus, there is focal repetitive interruption to the elastica in this area, analogous to the perforations in a postage stamp.

Comment

The normal characteristics of the elastica considered to be of most significance were the variability in the number of plates, the lack of evidence of an internal elastic lamella, and the occurrence of "pseudofragmentation" without apparent damage. The failure to demonstrate an internal elastic lamella is at variance with other reports.[25] Admittedly, this layer was distinguished with difficulty, and one might term the first elastic plate an internal elastic lamella, but it usually appeared no more prominent than any other plate. Another significant finding was the relatively high incidence of damage to the elastica by atherosclerosis even in this area of the aorta, supposedly least susceptible to the process.[19] Indeed, a perusal of the gross descriptions of the most severely affected aortas indicated that the degree of damage was frequently not appreciated grossly. The association of damage to the wall of the aorta of any type with accumulation of acid mucopolysaccharide was likewise significant. Damage to elastica was not necessarily extensive for this to occur, since the substance was prominent in superficial but early atheromatous plaques.

Histochemical study indicates the acid mucopolysaccharide was largely chondroitinsulfate C. The following characteristics were seen:

(*a*) Metachromasia at pH 4.0 and 6.0, but not at 2.0, indicating an incompletely sulfated compound. Increase of metachromasia at pH 2.0 after sulfation tended to confirm this.

(*b*) Metachromasia was reversible by methylation but could not subsequently be restored by KOH saponification. This indicated a sulfated acid mucopolysaccharide.[23,26]

(*c*) The substance was largely labile to testicular, but not to streptococcal, hyaluronidase.[6,7]

These characteristics of the metachromatic substance were identical regardless of the quantity present. They were also consistent with chemical data indicating a predominance of chondroitinsulfate.[6,7,20] The substance also stained in a positive manner with the Alcian blue stain and in negative fashion with both the PAS and coupled tetrazonium reactions. The negative reaction with the PAS stain coincides with recent observations concerning the staining characteristics of acid mucopolysaccharides.[30] There was wide variation in the quantity of the acid mucopolysaccharide in the absence of other evidence of abnormality, but the substance tended to accumulate in the vicinity of atheromatous plaques and areas of damage to the aorta (including one with syphilitic aortitis). The deposit was prominent in instances of early damage whatever the cause; later, it was replaced by collagen. It did not vary quantitatively with age, thus controverting the observations of Bunting and Bunting,[9] but agreeing with those of Kirk and co-workers.[20] It is possible that the former study, dealing largely with a lower segment of the aorta, where arteriosclerosis is usually severer,[19] was unduly influenced by this phenomenon.

The only circumstance in which there was reduction of acid mucopolysaccharide (other than normal variation) was in the case of the acellular linear foci presumably representing areas of loss of both smooth muscle and fibrocytes. Disappearance of metachromatic material from these areas was characteristic, but the significance of this observation is not clear. It could be taken to indicate that the cells producing the mucoid material were no longer present; on the other hand, if the chromotropic substance served a nutritive function, its own disappearance could conceivably be followed by death of cells in the affected region.

The smooth muscle component contributed a relatively small proportion to the wall of the aorta. The vessel is predominantly an elastic artery, and it would appear far-fetched to attribute a major functional role to the smooth muscle, especially in view of the great success recently attained with transplantation of acellular aortic segments.[31] Nevertheless, Gsell,[32] Rottino,[33,34] and Gore,[18] in many cases of dissecting aneurysm, have attributed the lesion to defects in muscle. Rottino[33,34] described muscle defects resembling the acellular foci noted in our aortas in 95 of the 210 aortas he examined. It is not clear from his survey whether this observation included such foci in contiguity with areas of atherosclerosis. If the present study had included such lesions in this category, the incidence might have approached that indicated by Rottino. However, it appears more logical to consider such foci to be a concomitant of arteriosclerosis. The cause and significance of the acellular linear foci within the media and not associated with arteriosclerosis are unapparent. There did not appear to be any significant association with hypertension. In view of the apparent lack of importance of the cellular component of the aorta, it is considered unlikely that they can be of significance in the pathogenesis of dissecting aneurysm. The apparent high frequency of their occurrence in random necropsy populations requires caution in assessing the importance of their presence in the cases of dissecting aneurysm. Furthermore, if a simple sampling technique indicates an incidence of these lesions as high as 7.5%, it may be that this alteration is quite commonplace. The question must be raised as to whether these are actually acquired abnormalities or represent anomalous defects occurring in a large proportion of essentially normal aortas.

The connective tissue component, like the acid mucopolysaccharide, may be considered a filler substance. Evidence indicates that in early damage to the wall of the aorta, acid mucopolysaccharide appears, to be replaced subsequently by connective tissue. This phenomenon has been observed in other

areas of damage followed by repair (granulation tissue).[35]

The survey indicates a surprisingly high incidence of severe sclerosis of the ascending aorta. The disproportionate frequency of fibrous plaques and medial calcification probably accounted for the failures to recognize the severity of the lesion grossly. Frequently, the elastica was extensively damaged by the extension of plaques. Ulceration occurred often, and in four instances short tracts extended from the intima into the media through ulcerated plaques. This type of lesion could conceivably serve as a portal of entry for a column of blood, the blood then dissecting along the natural defects in the elastica created by the perforating, ramifying vasa vasorum.

Summary and Conclusions

Sections representing the entire length of ascending thoracic aorta obtained at necropsy from 200 unselected adults were studied histologically and histochemically.

The normal structural variations of the elastica were elucidated.

There was considerable normal variation in quantity of acid mucopolysaccharide; this substance apparently consists largely of chondroitinsulfate C and is present in free form in large quantities, tending to increase in areas of damage to the elastica and in early atheromatous plaques.

Severe arteriosclerosis, commonly unrecognized on gross examination, was surprisingly frequent. Marked damage to the elastica resulting from extension of such plaques was common. A high proportion of the plaques were of fibrous rather than atheromatous nature.

Minute defects in the smooth muscle component were detected with moderate frequency (7.5%), but neither their cause nor their significance could be ascertained.

The implications of these observations with respect to the pathogenesis of dissecting aneurysm is briefly considered.

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Generalized Scleroderma Involving Lungs and Liver with Pulmonary Adenocarcinoma

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Five cases of scleroderma associated with carcinoma of the lung have been reported in the literature. This report adds a sixth case and emphasizes the relationship of pulmonary fibrosis to carcinoma. In addition, apparent evidence of hepatic involvement associated with systemic scleroderma is presented.

Report of a Case

A 57-year-old white housewife was readmitted to the University of Michigan Hospital on Feb. 6, 1957, complaining of shortness of breath and pain in the left side of the chest.

The patient had been seen first at the University Hospital in July, 1955, with wheezing, cough, and dyspnea of one year's duration and of dysphagia for four years.

She had been well until 1946, when she first had numbness, pain, and blanching of the fingers, diagnosed as Raynaud's disease. In 1949 a submucosal calcium deposit was removed from the floor of her mouth. Chest x-ray films during the first admission revealed densities at the bases of the lungs, compatible with sclerodermal changes. X-ray examination of the upper gastrointestinal tract showed absence of peristaltic activity in the lower third of the esophagus. The only gross skin changes at that time were thickening of the skin of the fingers and ulcers of the finger tips. Skin biopsy was compatible with early scleroderma.

The family history indicated that the patient's father, sister, and son had asthma.

The cutaneous lesions improved during treatment with para-aminobenzoic acid N.F., and she was relatively free of complaints until two weeks prior to the final admission.

Physical examination in 1957 revealed tight, thick skin over the fingers, but no generalized dermal atrophy. The lower left hemithorax was flat to percussion, with decreased breath sounds. There were diffuse rales and rhonchi throughout the lungs.

Blood studies revealed moderate leukocytosis. The serum Kahn reaction, heterophil agglutinations, total serum protein levels, albumin-globin ratio, electrophoretic pattern, and lupus-erythematosus-cell preparations were within normal limits. Chest x-ray films showed a left pleural effusion. Stenosis of the lower-lobe bronchus was demonstrated by bronchoscopy, but no mucosal lesions were seen: vital capacity was 1.6 liters, or 52% of the expected value. The patient became steadily more dyspneic and died 24 days after admission.

Gross Necropsy Findings

The body was that of a middle-aged, fairly well-nourished woman. The skin was generally thickened and edematous and showed none of the atrophic changes or loss of hair associated with advanced scleroderma. Aside from a small area of lentigo on the abdomen, there was no abnormal pigmentation or calcification. Healed pinpoint ulcers were present on all of the finger tips. The pectoral muscles were flabby and edematous, but there was no apparent atrophy. The heart weighed 300 gm. The myocardium was of the usual color and consistency, without evidence of fibrosis. The left pleural cavity contained 500 cc. of loculated serosanguineous fluid, and the pleural surfaces were studded with small neoplastic implants. The left lung weighed 1,300 gm., and the entire lower lobe was replaced by a solid mass of firm, white neoplasm, the cut surface of which had a thick, mucoid character. The right lung was grossly free of neoplasm. In both lungs there were patchy areas of induration, especially at the periphery. The remaining parenchyma had a fibrotic texture. No endobronchial neoplasm was demonstrated. The peritoneal cavity contained 200 mm. of clear yellow ascitic fluid. The liver weighed 2,200 gm. The surface was finely granular, and the parenchyma had a leathery consistency. There was a solitary metastasis in the liver. The right adrenal gland contained a single metastasis and also a cortical adenoma. No abnormalities in the thyroid, spleen, pancreas, or kidneys were noted. The esophagus was not stenotic, and the wall was soft and edematous; fibrosis was detected. The stomach, small intestine, and colon had walls of normal

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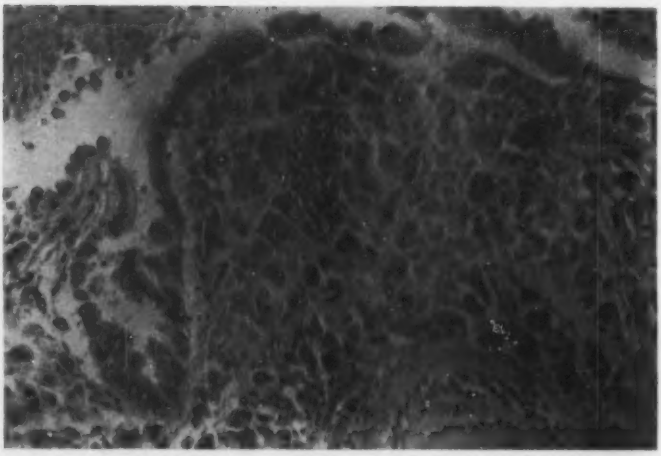

Fig. 1.—Anaplastic epithelium adjacent to pleomorphic carcinoma, bizarre cells. Hematoxylin and eosin; reduced to 63% of mag. × 380.

thickness, and no edema or fibrosis was demonstrated.

Microscopic Findings

The lungs showed advanced interstitial fibrosis with epithelization of alveolar lining cells. This process was extensive but did not involve the entire lung on either side. Sections from the left lower lobe showed diffuse invasion by poorly differentiated and pleomorphic carcinoma, which tended to form gland-like structures in some areas. Transitions between the atypical alveolar metaplasia and the neoplasm were evident (Figs. 1 and 2).

Metastases in the liver, serosa of the stomach and duodenum, adrenal gland, and kidney had a similar appearance (Fig. 3).

In the liver there was a marked concentric fibrosis (Fig. 4) about the bile ducts. Lymphocytes were present in increased numbers in the portal areas, as were a few eosinophils. There was no evidence of bile. The gallbladder was thin-walled, without evidence of chronic cholecystitis.

The epidermis of the abdominal skin was somewhat atrophic, but within normal limits for the age of the patient. The dermis was thickened, and the collagen fibers were thick and hyalinized.

Submucosal fibrosis of the esophagus was striking, particularly in the lower third, but the remainder of the gastrointestinal tract,

Fig. 2.—Atypical peripheral bronchiolar epithelium. Fibrosis of adjacent pulmonary parenchyma. Foci similar to this were abundant at the periphery of the neoplasm. Hematoxylin and eosin; reduced to 63% of mag. × 380.

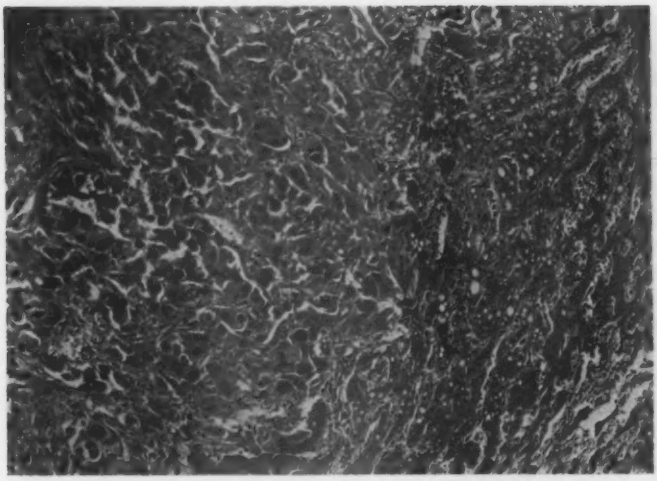

Fig. 3.—Poorly differentiated metastatic carcinoma in the liver, giant nuclei. Hematoxylin and eosin; reduced to 61% of mag. × 175.

except the colon, and the kidneys, showed no sclerodermatous changes.

Final Pathologic Diagnosis

Scleroderma, involving skin, lungs, esophagus, colon, pericardium, and liver. Atrophy of diaphragm and laryngeal skeletal muscle. Anaplastic bronchiolar-cell carcinoma of lung with metastases to right adrenal gland, kidney, liver, stomach, and duodenum. Ascites. Left hydrothorax. Hydropericardium. Pulmonary arterial emboli. Degenerative fatty infiltration of liver. Cortical adenoma of right adrenal gland. Thyroid adenoma.

Comment

Zatuchni et al.[1] in 1953 presented three cases of scleroderma in women with associated interstitial pulmonary fibrosis and terminal bronchiolar carcinoma. At that time, the association of cancer and scleroderma had not previously been reported, although Zarafonetis had observed 3 additional cancers in 39 patients with scleroderma, 2 of which were carcinomas, the other a fibrosarcoma.

The above report was followed in 1956 by that of Jonsson and Houser.[2] The authors described a 32-year-old woman with a 10-

Fig. 4.—Marked periportal fibrosis in liver with focal chronic inflammatory reaction. No evidence of cholangitis. Hematoxylin and eosin; reduced to 63% of mag. × 175.

year history of scleroderma, who developed pulmonary fibrosis, followed by poorly differentiated adenocarcinoma.

Collins et al.[3] in 1958, reported an additional case of bronchiolar-cell carcinoma in a patient with scleroderma and "honeycomb" lung.

No example of associated scleroderma and carcinoma of the lung, in the absence of pulmonary fibrosis, has been reported. If there is a causal relationship, the carcinoma is probably related to the pulmonary fibrosis rather than to scleroderma per se. In the present case, the histologic association between the adenocarcinoma and the pulmonary fibrosis was so striking that in some fields neoplasm could not be distinguished with certainty from hyperplastic, atypical, and incipient carcinomatous change in alveolar cells.

The implication that alveolar- or bronchiolar-cell carcinoma develops from epithelial hyperplasia occurring in chronic inflammatory foci, or that pulmonary tumors may result from stimulation of bronchiolar and/or alveolar lining cells in interstitial fibrosis, is not a new one.[4-8] As Bell stated: "Hyperplasia may give rise to localized or diffuse adenomatous growth which may even have the ability to metastasize."[4]

Beaver and Shapiro[9] proposed that terminal bronchiolar carcinomas arise from epithelized alveolar cells in areas of fibrosis or chronic pulmonary parenchymal inflammation. On the basis of their experience, they expressed the belief that the two processes were intimately related, and that the alveolar epithelization or bronchial metaplasia secondary to the chronic process may represent the precursors of carcinoma.

In their study, Watson and Smith[10] concluded that preexisting pulmonary disease is frequently demonstrable in patients who develop bronchiolar carcinoma.

It appears that pulmonary fibrosis, regardless of cause, at least in the peripheral carcinomas and bronchiolar carcinomas, may play a significant role in the pathogenesis of such lesions. In accord with this, the "scar" concept and relation of infarcts to pulmonary carcinoma have recently been reemphasized.[11] Freidrich[12] suggested that the genesis of these lesions may be similar to that of hepatocellular carcinoma in a cirrhotic liver.

The origin of pulmonary fibrosis in many cases is not clear, but it is recognized that the lungs possess a limited capacity to react specifically to infections or to injury caused by noxious agents.[13]

The principal predisposing factor is apparently fixation of lung tissue, whether from fibrosis, atelectasis, or immediate juxtaposition to infarcts or abscesses.[6] This is followed by anoxemia and loss of respiratory function due to interalveolar fibrosis and thickening. A reactive proliferation of epithelium occurs as a consequence. This proliferation on occasion crosses the "line" between metaplasia and neoplasia.

That a similar sequence may easily occur in the case of pulmonary fibrosis due to scleroderma is suggested in this report and that of Zatuchni and associates.

Getzowa's[14] classic description of the pulmonary changes and the experience of others[15,16] attest to the degree of fibrosis that can be reached in pulmonary scleroderma.

Although the incidence of malignancy in patients with scleroderma is not as prominent as in dermatomyositis,[17] the association of peripheral adenocarcinoma of the lung with sclerodermal pulmonary changes is higher than would be expected by chance alone.

The biliary-tract changes in this patient are interpreted as a manifestation of scleroderma, since there was no evidence of malnutrition, previous hepatic or biliary disease, chronic passive congestion, intestinal defect, or ulcerative colitis. Since the biliary system is an outgrowth of the primitive gut, it is reasonable to suspect that such extensive periductal fibrosis, particularly in the absence of concurrent evidence of biliary-tract obstruction, might correspond to the submucosal fibrosis commonly seen elsewhere

in the gastrointestinal tract in this disease. In spite of the widespread visceral involvement attributed to generalized scleroderma, only a few vague references to associated liver disease could be found in the literature.

Goetz,[18] in a detailed study of many visceral lesions in a 42-year-old white woman with scleroderma of 14 years' duration, briefly mentioned biliary cirrhosis as an incidental finding but presented only description without illustrations.

Harvier and Bonduelle[19] described a 43-year-old white woman with long-standing diffuse scleroderma associated with hereditary telangiectasia. Exacerbations of the scleroderma were preceded by recurrent jaundice. The author believed on clinical evidence that this represented cirrhosis related to scleroderma, but histologic confirmation was not available.

Milbradt[20] presented the case of a 47-year-old white woman with a diffuse scleroderma of 15 years' standing, associated with hereditary telangiectasia. Exacerbations of the scleroderma were preceded by recurrent jaundice. The author believed clinically that this represented cirrhosis related to scleroderma, but histologic confirmation was not offered.

Recently, Calvert et al.[21] presented two cases of systemic scleroderma with portal hypertension and varices associated with hepatic fibrosis. They postulated the possibility of scleroderma-induced portal hypertension.

Massimo[22] reported that hepatosplenomegaly should be included in the picture of systemic sclerosis and noted that hepatic involvement in scleroderma was not reported in the literature.

Summary

The sixth case of carcinoma of the lung associated with systemic sclerosis and pulmonary fibrosis is recorded. The rarity of coexistence of the two conditions is probably more than coincidental.

The possibility of hepatic fibrosis, at times reaching cirrhotic proportions, in scleroderma, and most likely directly attributable to scleroderma, is presented, with photomicrographic evidence.

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C^{14}-Labeled Bacterial Polysaccharide and Lymphosarcoma of the Rat

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Tumor necrosis following the injection of bacteria or bacterial products in man [1-4] and the dog,[1,5] guinea pig,[6] rat,[7,8] and mouse [8-13] has been known for many years. Although not extensive, studies on various tumors, animals, and bacterial products indicate that (*a*) rapidly growing, transplantable malignant tumors are most susceptible to necrosis,[7,11,13] and (*b*) lipopolysaccharide complexes [14,15] from certain Gram-negative bacteria have marked tumor-necrotizing properties. These bacterial complexes, usually toxic and antigenic, produce a variety of other biologic responses,[16,17] but the mechanism and interrelationships of these responses to tumor necrosis are obscure.

The marked uptake of C^{14}-labeled Klebsiella pneumoniae polysaccharide in the lymph node of the rat [18] led to the present study on the sequential localization of this bacterial substance in the Murphy-Sturm lymphosarcoma of this species. The observations of this study include (*a*) the delayed and moderate uptake of the C^{14} by the tumor, (*b*) weight changes of carcass, liver, and spleen with rapid tumor growth, (*c*) a partial extraction and immunologic identification of C^{14}-labeled material from the tumor, and (*d*) an estimation of possible tissue redistribution and/or degradation of the bacterial polysaccharide. These findings are correlated with prior observations of tumor regression induced by bacterial products.

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Materials and Methods

Murphy-Sturm lymphosarcoma * was propagated in young male Sprague-Dawley (Holtzmann) rats by subcutaneous injection over the lumbospinal muscles of approximately 100,000 cells suspended in Earle's solution. The rats weighed 90-110 gm. at the initiation of the experiment and were maintained on Purina Laboratory Chow and water ad libitum, with daily supplements of leafy vegetables. Animals were weighed each day, and after the appearance of tumor, on the eighth or the ninth day, the maximum and minimum diameters of the tumor were recorded daily. Tumor weight prior to autopsy was estimated from a plot of average tumor diameter to actual tumor weight. Since lethal effect of bacterial polysaccharide was proportional to tumor size, only rats with tumors of 10-20 gm. were used in the present study. One-third of each group of tumor-bearing rats were maintained as controls for spontaneous regression.

The C^{14}-labeled K. pneumoniae polysaccharide, prepared biosynthetically from media containing sodium acetate-1-C^{14}, as previously described,[19] had a specific activity of 0.98μc/mg. It was haptenic and nonlethal in control rats.[18] The bacterial polysaccharide, as a 1% solution in 0.85% sodium chloride, was injected by tail vein, 0.5 mg/100 gm. total body weight. At various intervals after the injection of the polysaccharide, the rats were anesthetized with ether and exsanguinated by cardiac puncture. Study of C^{14} uptake beyond one week after polysaccharide injection was possible only in those animals with regressing tumors, since animals with the progressively growing tumor died within this period. At autopsy, tumor, liver, spleen, and left adrenal were removed and weighed, and samples of these tissues, as well as the lung, kidney, thymus, and mesenteric lymph nodes, were ground in a glass homogenizer, diluted with water, aliquants plated, and C^{14} determined with a windowless flow counter. The C^{14} content of plasma obtained by centrifugation from heparinized blood

* Obtained from H. C. Stoerk, Merck Institute for Therapeutic Research, Rahway, N.J.

was determined similarly, and the total C^{14} in plasma was based on the plasma volume as 4% of body weight. Results are expressed as (*a*) per cent of injected C^{14} in the total plasma, liver, spleen, and adrenal, and (*b*) per cent, per gram dry weight, in these and other tissues. Portions of tumors from animals killed three days after the injection of labeled polysaccharide were pooled, ground, and extracted, and precipitin tests of the extracts with anti-Klebsiella serum were performed, as previously reported.[18-20]

To estimate the uptake of C^{14} from labeled polysaccharide into the tumor either from the "general carbon pool" or by redistribution from various tissue sites into the rapidly growing tumor, labeled polysaccharide was injected intravenously, 0.5 mg/100 gm. body weight, three days after tumor implantation and before any palpable or visible growth of tumor had occurred. The C^{14} in various tissues of these rats was determined 7 days after polysaccharide injection (10 days after tumor implantation). Normal rats of the same body weight as tumor-bearing rats were injected with the same quantity of labeled polysaccharide and the C^{14} uptake determinated seven days after injection as described above.

Histologic sections of the tumor and other tissues were stained with hematoxylin and eosin.

Results

After the appearance of the tumor on the eighth or ninth day following transplant, the tumors had a rapid and approximately linear growth, with accompanying decline in body (less tumor) weight (Fig. 1). The weight of the liver was maintained relative to the body (less tumor) weight and did not increase with the growing tumor mass (Fig. 2). The relative weight of the spleen initially

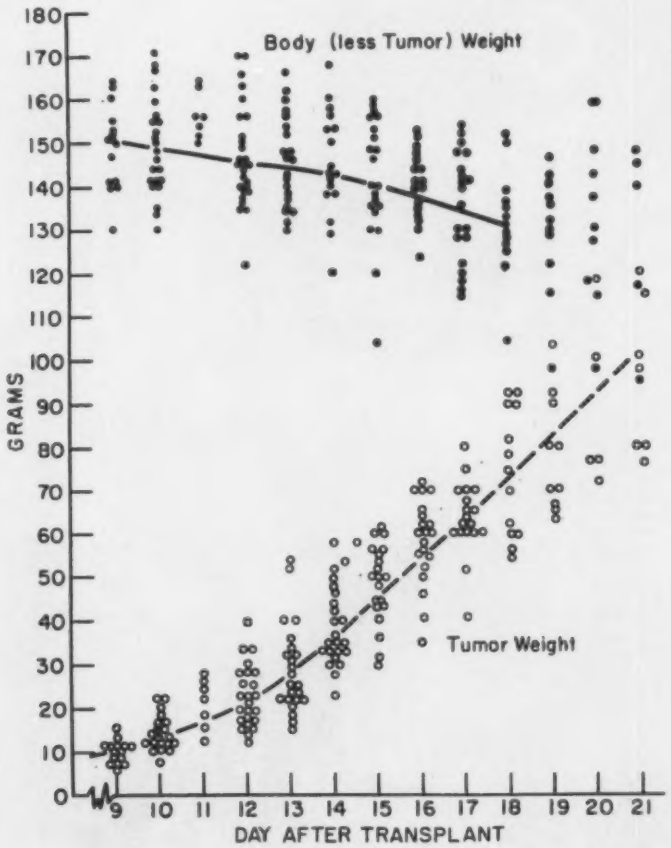

Fig. 1.—Loss of carcass weight with rapid tumor growth in young rats.

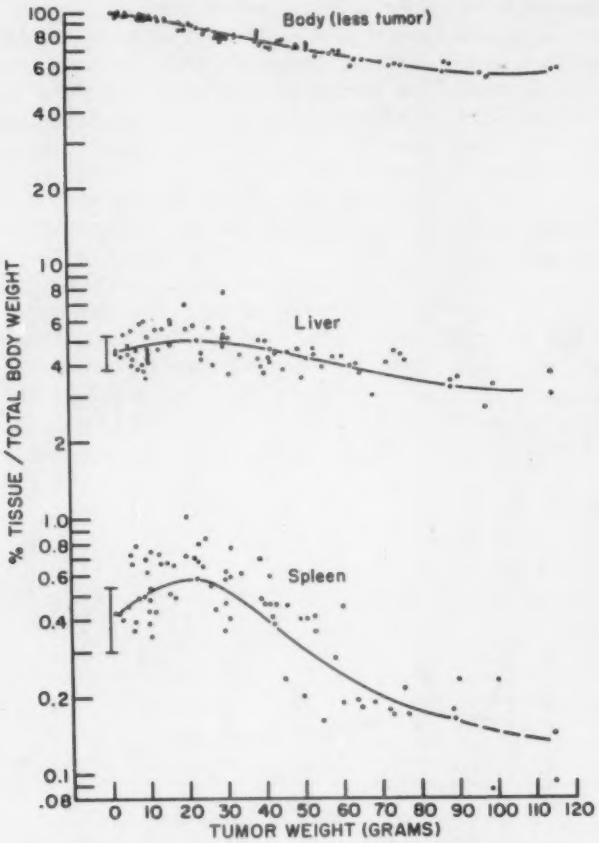

Fig. 2.—Semilogarithmic plot of carcass, liver, and spleen weights as the per cent of total body weight (carcass plus tumor); normal controls are indicated by vertical bracket. Liver weight initially increased with tumor growth, but later, with marked increase in tumor weight, the relative liver weight decreased. Parallel slopes of relative liver and carcass weights indicate that ratio of liver weight to carcass weight remained unchanged even with massive tumor growth. The relative weight of the spleen initially increased, but later decreased far more than the liver or carcass.

increased and then, with the more marked growth of the tumor, decreased to less than that of normal controls (Fig. 2). In addition, the adrenal weight was maintained relative to total body weight, while the changes in thymic mass paralleled changes of the spleen.

Spontaneous regression of tumors occurred in 8% to 12% of the animals and usually began when tumors were 10-15 gm. in weight. Approximately 30% of animals with tumors weighing 10-20 gm. died during the first 12 hours following the injection of bacterial polysaccharide and are not included in this study. Effects of polysaccharide upon tumors in surviving rats were almost equally divided into three categories, (*a*) regression, (*b*) transient growth arrest, and (*c*) no effect (progressive growth).

Within a day after the injection of the polysaccharide, the tumor became even more spheroidal. Serum sometimes oozed from the overlying cutaneous surface, and, when transected, the tumor showed an increase in the congestion of tumors of this size, as well as focal hemorrhages and surrounding protein-rich exudate. By the second or third day, large, irregular, more or less central areas of coagulation necrosis or smaller, scattered zones of necrosis were evident. In regressing tumors, peripheral replacement of the coagulation necrosis by polymorphonucelar leukocytes, macrophages, fibroblasts, and capillaries was prominent at one week. In tumors with partial necrosis the viable

and growing tumor usually appeared at the edges of the original tumor. Continued growth of the tumor resulted in marked extension about the retroperitoneal structures, such as the kidney and adrenal, occasional metastases to inguinal nodes, and infrequent extension to the bowel, mesentery, porta hepatis, and omentum.

C^{14} from the labeled polysaccharide appeared in the tumor more slowly than in the liver, kidney, and lung, but this delayed uptake was less marked than in the mesenteric lymph nodes (Figs. 4 and 5). At 24 hours the C^{14} concentration in the tumor was comparable to that in the liver, spleen, and adrenal (Figs. 4 and 5), and the total C^{14} in the tumor was similar to that in the liver (Fig. 3), which the tumor approximated in mass. The total C^{14} in the lymphosarcoma remained at about the same level for the first seven days regardless of whether necrosis or progressive growth had occurred (Fig. 3). Separate plotting of the areas of necrosis and those of viable, growing tissue (Fig. 4) disclosed a decrease in C^{14} concentration per unit weight with the continued growth of the tumor, while the concentration in necrotic tissue persisted or increased until the ninth day after the injection of bacterial polysaccharide. At this time only regressing, necrotic tumors were available for study. The decreased concentration of C^{14} from the 9th to 14th days in regressing tumors was accompanied by

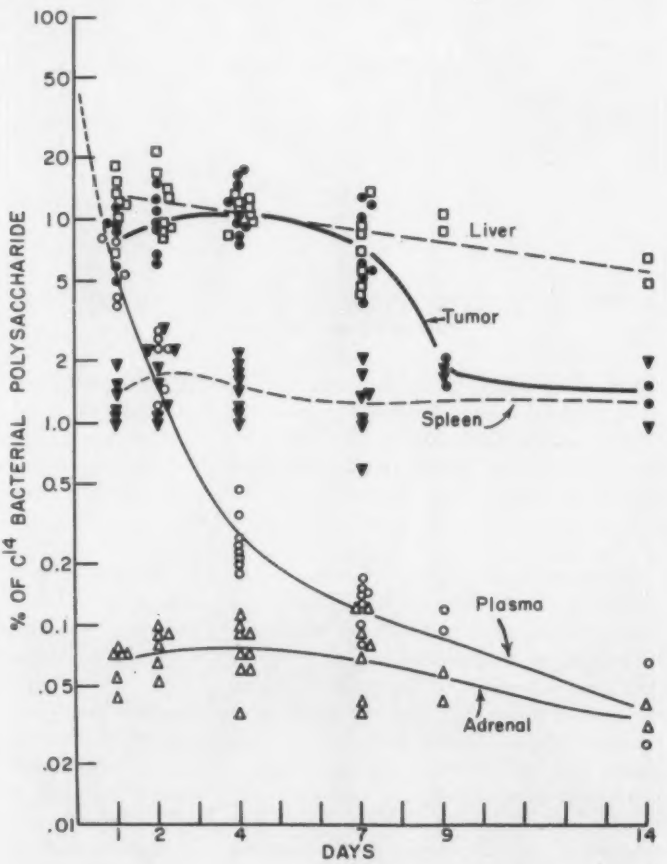

Fig. 3.—The uptake of C^{14} from labeled K. pneumoniae polysaccharide, expressed as the per cent of injected material in the liver, tumor, spleen, and adrenals as compared with the per cent remaining in the plasma. Despite tumor growth, the total quantity of labeled material within the tumor remained almost constant up to death at or near seven days after injection of bacterial polysaccharide. Values for 9 and 14 days for rats with necrotic tumors only.

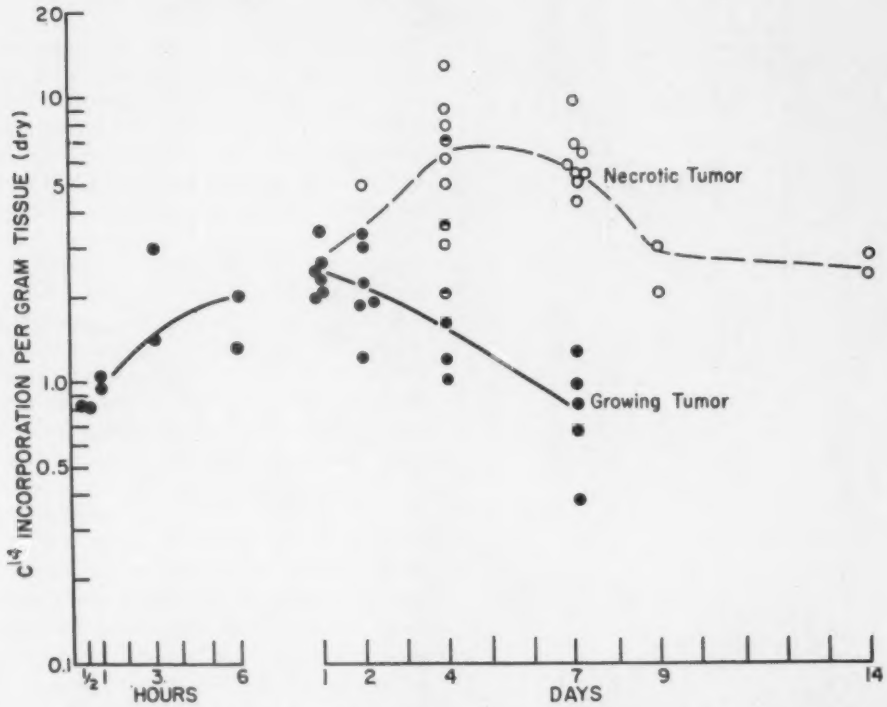

Fig. 4.—Uptake of C^{14} from labeled K. pneumoniae polysaccharide as the per cent of injected dose per gram (dry weight) of tumor tissue; concentration was decreased in tumor with progressive growth (solid line of solid circles), was increased in necrotic tumor (line of clear circles), and was intermediate in tumors with closely intermingled areas of necrotic and intact tissue (line of half-clear and half-solid circles).

histological evidence of replacement of necrotic tumor.

As in controls previously reported [18] and those observed in this study (Table 1), the concentration of polysaccharide was most marked in the mesenteric lymph nodes (Fig. 5). The C^{14} concentration in the lung and kidney declined at an approximately exponential rate, in contrast to the apparent retention in other tissues (Fig. 5). Some of the unusually high C^{14} concentrations in the spleen (Fig. 5) were attributable to the decreasing size of this organ with increasing tumor growth (Fig. 2); the total quantity of C^{14} in the spleen was similar in animals with both progressing or regressing tumors. The variability, both in tumor growth after injection of labeled polysaccharide and in C^{14} content in different organs, prevented an accurate estimation of relative or absolute increase in the tissue content of C^{14} during the phase of active removal of necrotic tumors.

In animals injected with the labeled bacterial polysaccharide before grossly detectable tumor growth (three days after transplant), the C^{14} in tumors was 20%-30% of that in tumors weighing 10-20 gm. at the time of injection (Table 1, Fig. 3).

C^{14}-labeled material with solubility characteristics of polysaccharide was extractable from tumors weighing 10-20 gm. at the time of injection of labeled bacterial polysaccharide. This fraction represented 18.4% of the C^{14} in the tumor (Table 2) and retained the haptenic properties of the original material in precipitin tests with anti-Klebsiella rabbit serum.

TABLE 1.—*Tissue Uptake of C^{14} Seven Days After Intravenous Injection of Labeled Klebsiella Pneumoniae Polysaccharide in Control Rats and in Rats Three Days After Tumor Transplant*

	Tumor		Liver		Spleen		Adrenal		Mesent. Lymph Node	Lung	Kidney	Thymus
	% per Gm.	% Total Tissue	% per Gm.	% Total Tissue	% per Gm.	% Total Tissue	% per Gm.	% Total Tissue	% per Gm.	% per Gm.	% per Gm.	% per Gm.
Control (8)			3.46 ±1.23	11.80 ±3.33	14.33 ±4.91	2.47 ± .79	2.20 ±1.11	0.032 ± .015	124.4 ± 88.2	0.54 ± .29	0.75 ± .12	0.67 ± .19
Before visible Tumor growth (3 Days after transplant)	0.29	1.83	3.95	8.29	5.05	1.23	5.29	0.09	250.7	1.74	0.85	0.59
	0.13	0.90	3.92	10.30	4.86	1.39	1.92	0.04	137.0	1.44	0.86	0.66
	0.36	2.30	2.66	10.60	4.87	2.52	3.07	0.06	--	1.00	0.67	0.93
	0.34	1.79	4.62	13.22	7.07	1.23	4.64	0.09	126.8	--	0.64	0.75

Comment

Seligman et al.[21] found relatively little radioactivity in mouse sarcoma after the intravenous injection of I^{131}-labeled Serratia marcescens polysaccharide. In contrast, the present study in the rat showed that the C^{14} from K. pneumoniae localized in high concentration and persisted in the Murphy-Sturm lymphosarcoma, as well as in the liver, spleen, and mesenteric lymph nodes, while disappearing from the plasma, kidney, and lung. The C^{14} in these tissues persisted despite the marked nutritional and metabolic demands of the rapidly growing tumor, with accompanying loss of non-neoplastic body weight. After incorporation into the tumor tissue, the label remained almost unchanged in quantity, decreasing in concentration with growth of the lymphosarcoma and disappearing from necrotic tumor with the active removal of the latter. The chemical nature of most of the labeled material in tissue has not been determined, but that which was extractable by a relatively simple procedure retained the haptenic property of the original bacterial polysaccharide. The quantity recovered from the tumor was similar to that extracted from the liver and lymph nodes of the rat.[18]

The extent of metabolic inertness and/or fixation of the bacterial polysaccharides in tissues was afforded by the animals receiving injections of the labeled material when the tumors were of microscopic size. Seven days later the markedly enlarged tumors contained approximately 20%-30% of the C^{14} found in tumors of animals injected when tumors were 10 to 20 gm. in weight. Further studies are necessary to determine whether the labeled material in the tumor was the result of the redistribution of the bacterial polysaccharide from tissue sites into the growing neoplasm or of the utilization of labeled moieties derived from the degradation of the polysaccharide.

Although the pathogenesis of polysaccharide-induced tumor necrosis is speculative, several observations warrant an attempt at

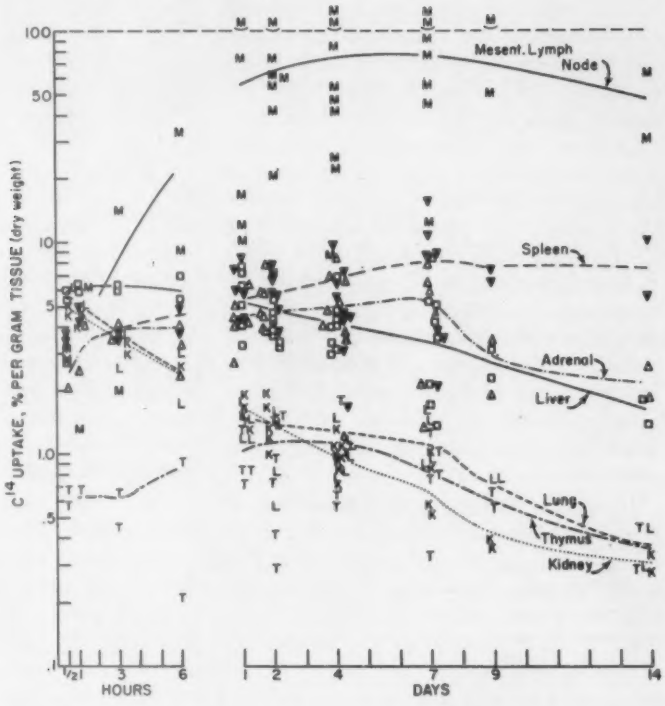

Fig. 5.—Uptake of C^{14} from labeled K. pneumoniae polysaccharide by various tissues as per cent of injected dose per gram of tissue (dry weight).

correlation with the distribution of C^{14}-labeled K. pneumoniae polysaccharide.

1. In vitro, tumor cells apparently are not injured by bacterial products.[29-32]
2. Rapidly growing tumors are most susceptible to necrosis.[7,11,19]
3. Tumor injury, in vivo, is manifested by edema, plasma exudation, hemorrhages, cytologic changes, and necrosis.[11,26-28]
4. Lethal toxicity of bacterial products is enhanced in tumor-bearing animals.[7-19]
5. Bacterial polysaccharide complexes are inactivated by plasma proteins.[31,32,33]

In simplest terms, bacterial products injure tumor tissue directly or indirectly. The evidence of direct effect of bacterial products in vitro upon tumor tissue, reported by Fogg,[34] was refuted by Shapiro,[22] McConnell et al.,[23] Zahl,[24] and Lasfargues et al.[25] Since the tumor tissue in vivo is injured by bacterial products, and since hemorrhage is a prominent manifestation of such injury, it is not surprising that the blood vessels have been considered the site of primary damage.[1,35,36] The delicate, thin-walled vessels of rapidly growing tumors may be prone to injury by bacterial products,[28] although apparently resistant to other hemorrhagic agents.[11] However, an initial injury to tumor cells could produce the local vascular changes.[11,26-28]

TABLE 2.—*Extraction of C^{14}-Labeled Material from Tumor Tissue*

		Lymphosarcoma	Water-Soluble Fraction	Alkaline-Soluble Fraction	Ethanol-Insol. Water-Resoluble Fraction
C^{14}	μc.×10⁻³	119	88.9	45.6	21.9
	%	--	74.7	38.2	18.4

The enhanced lethal effect of bacterial polysaccharide in tumor-bearing animals may be additional evidence of early injury to tumor. According to Zahl,[24] the enhanced lethal toxicity was dependent upon coexistent tumor; extirpation of the tumor within a few hours after the injection of bacterial polysaccharides markedly reduced the mortality. A reasonable interpretation of this observation is that the initial injury to tumor, indicated by plasma exudation or hemorrhage, is closely associated with the release of toxic lethal agents into the blood stream. The reduction in ribonucleic acid and adenosinetriphosphate content of tumors after injection of bacterial polysaccharides[37] also suggests a possible injury to the tumor.[28]

Incubation of bacterial polysaccharides or related products with plasma, in vitro, reduces their capacity to evoke fever,[29,30] death,[31] tumor hemorrhage,[32] and plasma 17-hydroxycorticosteroid (17-OHCS) elevation.[20,33] In the guinea pig, the inactivation of the K. pneumoniae polysaccharide has been associated with binding to plasma protein.[20] Although a transient, toxic complex of plasma protein and bacterial polysaccharide, comparable to an "endogenous pyrogen,"[38,39] cannot be excluded, the injurious effects of the polysaccharide would appear to be present for only a short time after injection and prior to extensive binding with plasma protein. Such initial local injury to tumor tissue, predominantly within the first hour would be attributable to relatively minor quantities of toxic polysaccharide. Furthermore, the delayed localization of "inactivated" polysaccharide in the tumor may be the result of the prior local injury, and not the cause of further cellular damage.

Reaction of the guinea pig adrenal to bacterial polysaccharide affords an interesting analogy to the local injury, C^{14} uptake, and functional or metabolic changes in tumors. As in tumors of rats and mice, bacterial products readily induce hemorrhage in the adrenal of the guinea pig. The uptake of C^{14} from labeled polysaccharide is also marked and delayed.[18,19] Unpublished studies indicate that most of the label in the adrenal is present in compounds with the properties of the original polysaccharide. Pretreatment with cortisone but not with cortisone plus corticotropin, inhibited the C^{14} uptake,[40] supporting the idea that initial local injury or the degree of functional or metabolic activity modifies the uptake of labeled polysaccharides. Following the injection of labeled polysaccharide, the transient plasma 17-OHCS elevation returned to control values and subsequently, despite the high concentration of labeled polysaccharide in the adrenal cortex, altered 17-hydroxycorticosteroidogenesis could not be demonstrated.[33] Thus, it appears probable that bacterial polysaccharide in tissue cells is "detoxified" and does not significantly modify cellular function.

The marked affinity of the polysaccharide for nucleic acids of the tumor and other tissues observed during the extraction of polysaccharide (unpublished studies) suggests that the binding of polysaccharide to intracellular components may be related to both the initial injurious effects and the subsequent inactivation without appreciable isotopic loss. While the present study on the distribution and disappearance of C^{14}-labeled K. pneumoniae polysaccharide or derivatives in the Murphy-Sturm lymphosarcoma has not disclosed the mechanism of induced tumor necrosis, several promising avenues of approach to this problem have resulted.

Summary

After intravenous injection, C^{14}-labeled polysaccharide from K. pneumoniae was found in Murphy-Sturm lymphosarcoma of rats in concentration similar to that in liver and spleen. C^{14}-labeled material extracted from the tumor retained the haptenic property of the original bacterial polysaccharide. The label persisted in tumors, decreasing in concentration in growing tumors and diminishing in regressing tumors with disappearance of necrotic tissue. The presence of tumor tissue did not modify the total uptake

of C^{14} in the liver and spleen, but some variation in concentration of C^{14} in the spleen and lymph nodes accompanied the atrophy of these tissues with massive tumor growth. The apparent metabolic inertness and fixation of the bacterial polysaccharide in tissues was supported by the mild tumor uptake of C^{14} from labeled polysaccharide, injected prior to the onset of palpable tumor growth.

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Occurrence of Osteoporosis in Mice with Muscular Dystrophy

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In preliminary experiments on the histopathology of Dystrophia muscularis, a mutant of Strain 129 mice with inherited muscular dystrophy (Michelson et al.[1]), osteoporotic changes were observed in the tibia of dystrophic animals killed at 4 months of age. The present experiment was undertaken to study the developmental changes in the bones of dystrophic mice at graded periods after weaning and to compare the observed findings with those of nondystrophic litter mates.

Experimental Procedure

Thirty dystrophic male mice (each derived from a different litter) and thirty nondystrophic litter mates were selected at approximately 5 weeks of age for the present experiment.* Animals were placed in cages containing one dystrophic mouse and its nondystrophic litter mate per cage and were fed a natural food stock ration† and water ad libitum. At 6 weeks of age four of the dystrophic mice and their nondystrophic litter mates were killed. Additional groups of four dystrophic mice and their nondystrophic litter mates were killed at 12 and 16 weeks of age and a group of five each at 20 weeks of age. Thirteen of the dystrophic mice died during the course of the experiment. At the time of killing, the heads, forelimbs, and hindlimbs were placed in neutral formalin. The specimens were decalcified in edathamil (Versene; EDTA) solution at pH 7.4, dehydrated, and infiltrated in the routine manner, embedded in 50% nitrocellulose, sectioned at 15μ to 20μ in thickness, and stained with hematoxylin and eosin.

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*Animals were obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

†Wayne Lab Blox, Allied Mills, Inc., Chicago.

Average Body Weight of Mice at Time of Killing

Age, Wk.	Dystrophic, Gm.	Nondystrophic Litter Mates, Gm.
6	14.6	20.6
12	19.3	26.4
16	19.1	26.6
20	18.5	25.1

Sections were also prepared with tissues embedded in paraffin and sectioned at 7μ.

Results and Comment

The body weight of mice in the various groups at the time of being killed is indicated in the accompanying Table. In agreement with preliminary findings, the tibia of dystrophic mice killed at 16 weeks of age exhibited severe osteoporosis and premature closure of the epiphyseal plate (Fig. 1). There was a reduction in the number of cells in the resting and proliferating regions. The hypertrophied and calcifying zones appeared to be absent and were replaced by a thin layer of lamellar bone, which sealed off the cartilage from the underlying diaphysis. There was complete loss of trabeculae in both the epiphysis and the diaphysis. Fat cells in the latter were present in abundance, occurring at the expense of the myeloid elements. These findings were in contrast to the normal microscopic appearance of nondystrophic litter mates of similar age (Fig. 2). Dystrophic mice killed at 20 weeks of age were similar in appearance to those in the 16-week series except that the number of fat cells in the tibia was increased (Fig. 3). Significant osteoporotic changes were also noted in the tibia of dystrophic mice killed at 12 weeks of age. In the latter, trabeculae were still present but were reduced to small

Figs. 1 to 4.—Photomicrographs of sections of the proximal epiphyseal region of the head of the tibia. *E.*, epiphysis; *E.P.*, epiphyseal plate; *D.*, diaphysis. Hematoxylin-eosin stain; × 50.

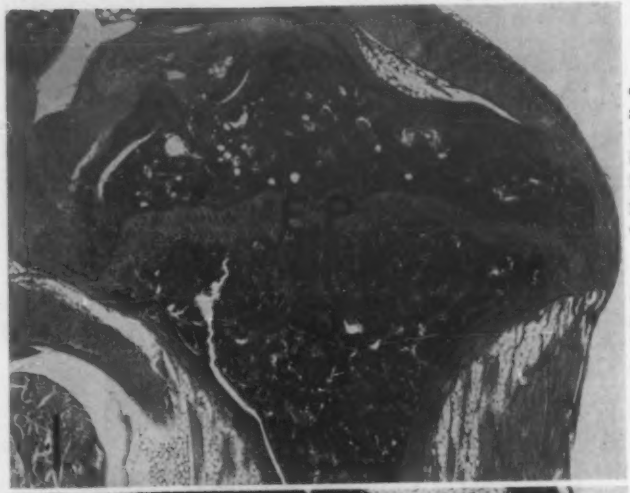

Fig. 1. — Epiphyseal area of the head of the tibia from a dystrophic mouse killed at 16 weeks of age. Note sealing off of epiphyseal plate by lamellar bone and osteoporosis of epiphysis and diaphysis.

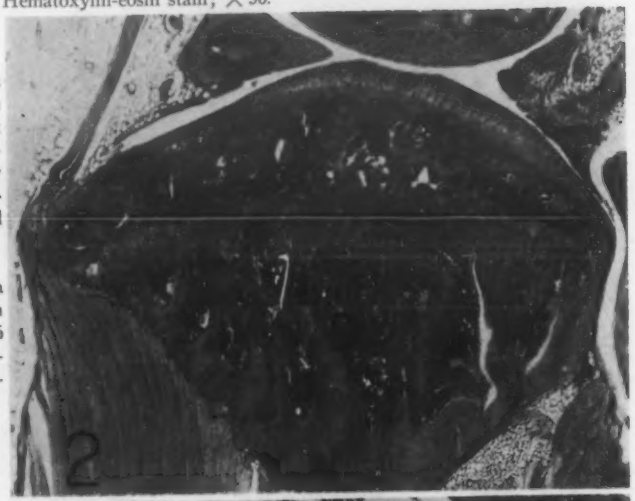

Fig. 2. — Epiphyseal area of the head of the tibia from a normal mouse killed at 16 weeks of age. Note well-developed trabeculae in epiphysis and diaphysis.

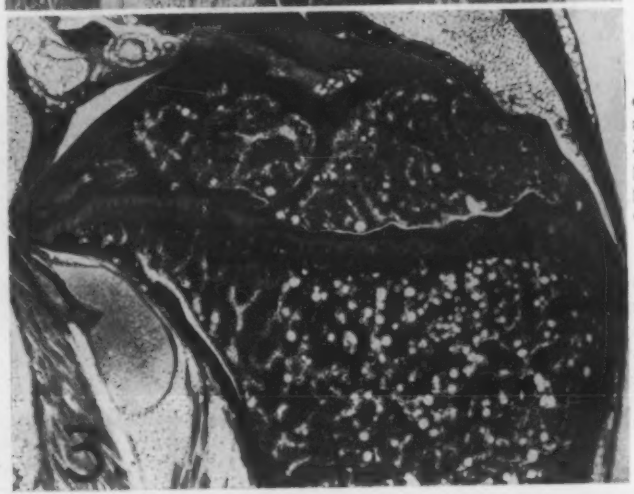

Fig. 3. — Epiphyseal area of the head of the tibia from a dystrophic mouse killed at 20 weeks of age. Note osteoporosis of epiphysis and diaphysis and fatty infiltration of marrow spaces.

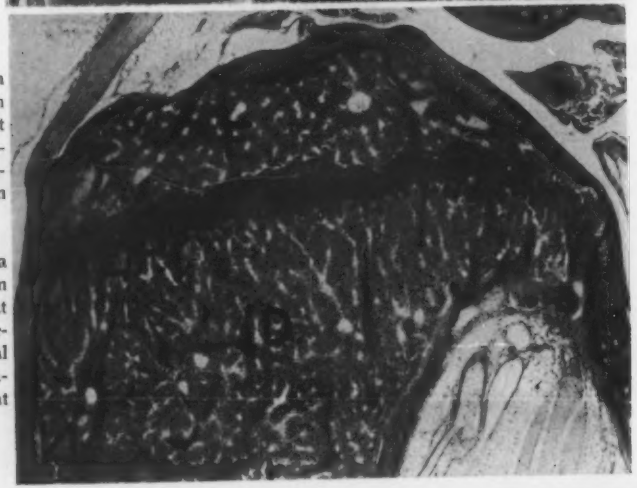

Fig. 4. — Epiphyseal area of the head of the tibia from a dystrophic mouse killed at 12 weeks of age. Note premature closure of epiphyseal plate, marked loss of trabeculae, and absence of fat cells in marrow.

Figs. 5 to 9.—Photomicrographs of sections of the gastrocenemius muscle. Hematoxylin-eosin stain; × 125.

Fig. 5.—Section of gastrocnemius muscle from dystrophic mouse killed at 6 weeks of age. Note loss of striations, destruction of muscle fibers, and infiltration of connective tissue.

Fig. 6.—Section of the gastrocnemius muscle from dystrophic mouse killed at 12 weeks of age. Note the beginning of fatty infiltration.

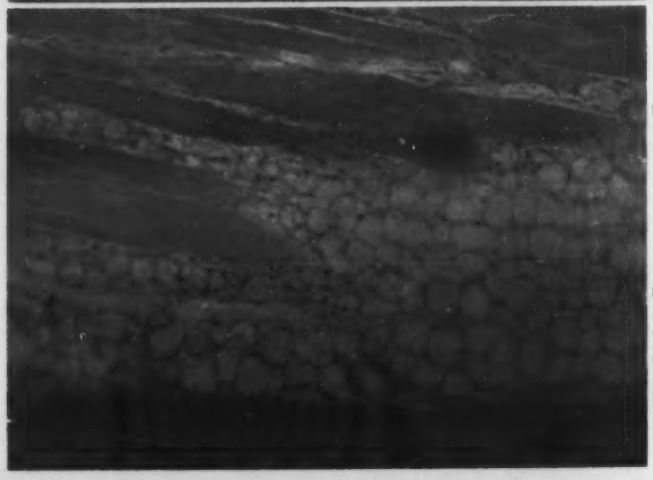

Fig. 7.—Section of the gastrocnemius muscle from dystrophic mouse killed at 20 weeks of age. Note further increase in fatty infiltration over 16-week stage.

Fig. 8.—Section of the gastrocnemius muscle from nondystrophic mouse killed at 16 weeks of age. Note normal appearance of fibers and absence of fat.

spicule-like processes. Fatty infiltration, however, was not manifest (Fig. 4). No abnormalities were observed in the microscopic appearance of the tibia of dystrophic mice killed at 6 weeks of age. In contrast to the pathologic changes in the tibia of dystrophic mice killed at 12 or 20 weeks of age, alveolar bone and the bone in the forelimbs of these animals had a normal microscopic appearance.

The gastrocnemius muscle of dystrophic mice 6, 12, and 20 weeks of age is illustrated in Figures 5, 6, and 7, respectively. Findings indicate a progressive increase with age in fiber destruction and infiltration of fat. In contrast, the gastrocnemius muscle of nondystrophic litter mates killed at the same time as the above were normal in appearance (Fig. 8). Clinically, in agreement with the findings of Michelson et al.,[1] symptoms of dystrophy as evidenced by occasional dragging of the hindlimbs and irregular gait were manifest as early as the 4th week of age. It is apparent from the above that the osteoporotic changes in the tibia occurred subsequent to clinical symptoms of dystrophy and the presence of microscopic lesions in the gastrocnemius muscle, and appeared about the time that fatty infiltration of the gastrocnemius muscle was first manifest.

Subsequent to the experiment indicated above, 20 dystrophic mice were selected at 16 weeks of age and were divided into two comparable groups of 10 animals each. One group received 10 daily intraperitoneal injections of 0.125 ml. of saline solution; the second group received 10 daily intraperitoneal injections of 0.125 ml. of growth hormone solution ‡ containing 25μg. of growth hormone. Twenty-four hours after the last injection, the mice were killed and the hindlimbs placed in neutral formalin. Sections were prepared as indicated above. No significant difference in response was observed between the two groups. Growth hormone, when administered under conditions of the present experiment, did not promote an increment in body weight. Histologically the tibia of mice administered growth hormone did not differ significantly from those of animals administered saline solution. Both groups were comparable in all respects to the dystrophic mice in the 16-week series of the previous experiment.

The cause of the osteoporotic changes indicated above is not readily apparent. It is well established that disuse produced by immobilization in plaster of Paris or nerve section results in bone atrophy (Grey and Carr,[2] Pottorf,[3] Allison and Brooks[4]). Since clinical symptoms of dystrophy preceded the onset of osteoporotic changes in the tibia of dystrophic mice, it is possible that the bone changes merely reflect disuse resulting from the progression of the disease. This explanation would appear tenable for the dystrophic mice killed at 16 or 20 weeks of age, for clinical symptoms of dystrophy were marked in these groups and were associated with virtually complete disuse of the hindlimbs. The dystrophic mice killed at 12 weeks of age, however, were clinically less severely affected and, although exhibiting occasional dragging of the hindlimbs, also exhibited periods of fairly normal locomotion. It would be difficult to account for the bone changes of these mice on the basis of disuse. Since reduction in blood supply caused by factors other than immobilization and nerve section can also result in bone atrophy (Bell[5]), further studies are indicated to determine to what extent alterations in blood flow due to conditions other than disuse may have contributed to the osteoporotic changes observed. Studies are also indicated to determine what relationship, if any, such changes may have on the course and progression of the disease process in the dystrophic mouse.

‡ Beef STH (Lot No. 728-074) was provided by Dr. Sanford L. Steelman, of the Armour Laboratories, Chicago. This material had an activity of 90%-100% of Armour Standard. When assayed on hypophysectomized female rats, 15μg. to 20μg. of the above material per rat per day, according to Dr. Steelman, produced a gain of 1 to 1.5 gm. per day. The powdered hormone material was suspended in water, and dilute alkali (NaOH) added to effect a solution. The volume was adjusted to a concentration of 200μg. per milliliter.

Summary

Premature closure of the epiphyseal plate and osteoporosis, with complete loss of trabeculae in the epiphysis and diaphysis, were observed in the tibia of Dystrophia muscularis, a mutant strain of 129 mice with inherited muscular dystrophy, when examined at 12, 16, or 20 weeks of age. Alveolar bone and the bones in the forelimbs of these animals, however, appeared normal. No abnormalities were observed in the tibia of nondystrophic litter mates killed at the same time as the above nor in dystrophic mice killed at 6 weeks of age.

Department of Biochemistry and Nutrition, University of Southern California School of Medicine, University Park (Dr. Ershoff).

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Natural History and Pathogenesis of Renal Ablation Hypertension

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Hypertension is known to follow subtotal nephrectomy in the rat. In previous studies from two-thirds to four-fifths of total kidney substance was removed.[1-3] Attention was called to the lesions which developed in the peripheral vessels and in the remaining renal stump.

In the present investigation hypertension was induced by simple resection of about three-quarters of the kidney substance. The pathological lesions, natural history, and pathogenesis of renal ablation hypertension were studied, especially the role of salt in the evolution of the disease.

Method

Young adult white male rats, weighing about 180 gm., were used. These were subjected to three-fourths renal ablation in one operative procedure by resecting the entire right kidney and the upper half of the left kidney. The latter was removed with a scalpel, and no attempt was made to control bleeding. The animals withstood surgery very well, and there was practically no mortality. The procedure resulted in necrosis of only a relatively small number of nephrons along the cut surface of the remaining renal stump. An organizing hematoma also developed along this surface but was not bulky and probably exerted no pressure on the parenchyma.

After operation the animals were divided at random into two groups as follows: (1) 30 rats, on standard diet of pellets and tap water for drinking; (2) 32 rats, on standard diet of pellets and 1% sodium chloride for drinking. Thus the animals in each group differed only in respect to their drinking fluid. The latter and food were offered ad libitum.

The weight and blood pressure of all rats were recorded weekly. Blood pressure was measured by the plethysmographic technique, and levels of 150 mm. Hg or over were considered hypertensive. There were control readings during the week before surgery.

At intervals a 24-hour quantitative urinary albumin measurement was made (Esbach method) in some rats. Blood urea nitrogen was determined before renal ablation and again at autopsy. The latter was performed after death or at time of killing, and tissues were taken from various organs for microscopic study. The heart was weighed after excising the atriae and removal of blood. The cardiac index was obtained from the formula

$$\frac{\text{Heart weight}}{\text{Body weight}} \times 100.$$

Results

Water Regimen.—Most of the rats with three-fourths renal ablation given tap water for drinking remained in fairly good condition and gained weight. They were killed at intervals of 7 to 10 months after surgery. A few rats died within 6 months, and several others were killed, in poor condition, between 7 and 10 months. The principal cause of death was pneumonia, usually associated with some degree of azotemia.

Sixteen per cent of the animals on water were hypertensive one week after renal ablation, and 40% were hypertensive at one month. Some rats did not develop high blood pressure until several months after surgery. The over-all incidence of hypertension was 56% (Table). The levels were usually in the 150-170 mm. Hg range, tended to be labile, and sometimes were not well sustained.

Vascular disease was observed only rarely in animals killed within one month after renal ablation. However, the incidence rose after this time and was 46% in rats studied between 5 and 10 months after ablation.

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From the Institute of Pathology, Western Reserve University School of Medicine.

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Incidence of Lesions

	Hypertension	Vascular Disease	Nephrosclerosis	Cardiac Lesions
Rats on water	56	46	77	20
Rats on salt	100	100	90	44

During the first few months following ablation, the renal stump showed changes which were essentially focal in nature. However, the lesions were clearly progressive, although at a variable rate, and eventually reached the stage of nephrosclerosis. The latter was characterized grossly by uniform granularity of the outer surface (Fig. 1). Microscopically moderate to severe nephrosclerosis was observed in 77% of animals autopsied from 5 to 10 months after renal ablation. Loss of protein in the urine often began within one month and was then progressive. From essentially zero control values, the amount excreted after several months by some rats exceeded 0.5 gm. daily. The blood urea nitrogen was essentially normal shortly after renal ablation was performed and then rose gradually to terminal levels of 46 to 105 mg. A rough correlation existed between the severity of the nephrosclerosis and the height of the blood urea nitrogen.

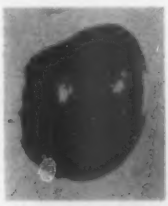

Fig. 1.—Nephrosclerosis of kidney stump eight months after three-fourths renal ablation.

There was a fairly close association of hypertension, vascular lesions, and renal disease, but this was not obligate. For example, a number of rats developed nephrosclerosis of the renal stump, but nevertheless remained normotensive.

Cardiac lesions were rarely observed within the first few months after renal ablation. However, they occurred in 20% of animals studied between 5 and 10 months.

Salt Regimen.—The rats with three-fourths ablation which drank 1% sodium chloride failed to gain weight at a rate comparable to that in rats on water, and growth was often permanently stunted. The life span was considerably shortened, and almost all the animals died within six months. The principal cause of death was pneumonia, accompanied by azotemia. Although there was usually no visible edema of soft tissues at autopsy, some rats showed free fluid in the peritoneal cavity.

Salt enhanced the development of hypertension. The latter occurred promptly, i.e., within one or two weeks, in practically all animals (Table). Usually the pressures were distinctly higher than for the rats with renal ablation on water (Fig. 2) and tended to be less labile and more sustained.

Salt also enhanced the development of vascular and renal disease. The lesions in the vessels appeared earlier and more frequently than in the animals drinking water. In ad-

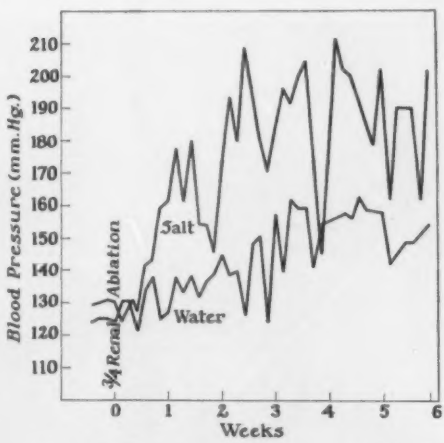

Fig. 2.—Hypertension following renal ablation. Curves represent the mean blood pressure of six rats drinking water and six drinking 1% sodium chloride.

dition, they were more widely distributed. Practically all rats on saline showed vascular disease after one month. Lesions of the kidney stump appeared earlier and progressed more rapidly in the rats on saline than in those on water. Well-developed nephrosclerosis was sometimes present within two months after ablation. Moderate or severe nephrosclerosis was observed in the great majority of animals which died between two and six months after renal ablation (Table). This was associated with elevation of the blood urea nitrogen (range 49-108 mg. %). Albuminuria was practically constant, sometimes occurred within one week after ablation, and often reached levels of 0.5 to 1.0 gm. daily within a few months.

Cardiac lesions were present as early as one or two weeks after ablation, and the incidence among animals studied between two and six months was 44%. During this period the mean cardiac index and its standard deviation were 0.42 ± 0.11. This corresponded to 0.38 ± 0.10 for hypertensive rats on a water regimen for a period of 5 to 10 months after ablation. The difference between the two groups was not statistically valid. However, each figure was significant in comparison with an index of 0.29 ± 0.07 for a group of normal rats [5] and indicated the presence of cardiac hypertrophy.

Sequence of Lesions

Some animals, from both the tap water and the salt groups, were killed from one to four weeks after renal ablation in order to study the peripheral vascular bed and renal stump at the onset of hypertension or shortly thereafter.

Vascular lesions were either absent or sparse. In the latter instance an occasional artery or arteriole showed such changes as slight periadventitial fibrosis, suggestive thickening and edema of the media, and/or early focal intramural fibrinoid necrosis. Apparently such lesions either coincided in time of origin with elevation of the blood pressure or began prior to it. A similar situation prevailed for the kidney stump. In most instances the latter was unaltered. Occasionally there was focal involvement of a few glomeruli and/or blood vessels.

Pathological Lesions

The lesions in the blood vessels, renal stump, and heart were the same for the rats on salt as for those on water.

Vascular Disease.—This was usually widespread, and the common sites included

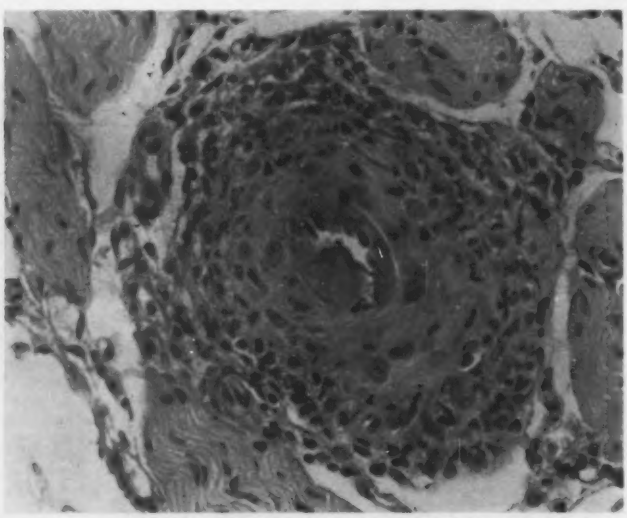

Fig. 3.—Necrotizing arteritis of small vessel in mesentery. Seven months after three-fourths renal ablation. Hematoxylin-eosin stain; ×250.

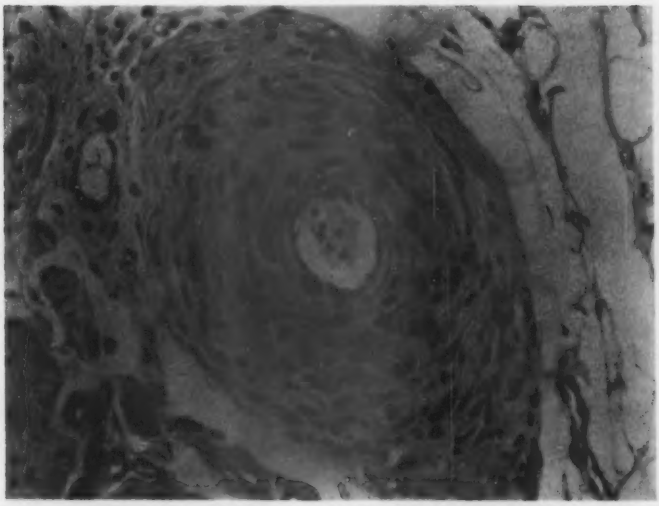

Fig. 4.—Proliferative arteritis of small vessel in mesentery. Seven months after three-fourths renal ablation. Hematoxylin-eosin stain; × 250.

pancreas, mesentery, gastrointestinal tract, adrenals, kidney stump, and testis. Arteries, small arteries, and arterioles were involved. The lesions often showed necrotizing, proliferative, and exudative components and hence resembled human polyarteritis (Fig. 3). However, there were variants, such as arteritis without necrosis, proliferative disease involving mainly adventitia and media (Fig. 4), and pure fibrinoid necrosis. Another change was thickening and edema of the medial coat with enlargement, altered polarity, and hyperchromism of nuclei, associated at times with cellular proliferation.

The lesions seemed to appear in crops during the hypertensive state. Healing was indicated by the presence of fibrosis or hyalinization of one or more layers of the vessel wall.

Kidney Stump.—There was early glomerular involvement, which was focal at first and then became widespread (Fig. 5). The basement membranes underwent thickening and degeneration, followed by adhesions be-

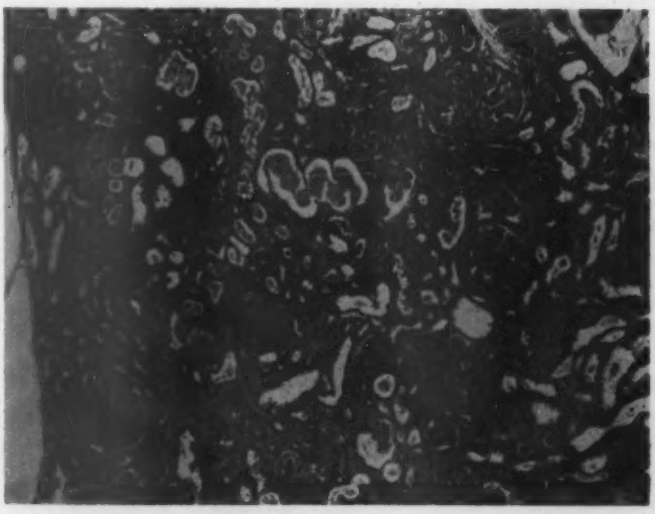

Fig. 5.—Nephrosclerosis in rat two months after three-fourths renal ablation. Drinking fluid was 1% sodium chloride. Note diffuse glomerular disease. Hematoxylin-eosin stain; × 40.

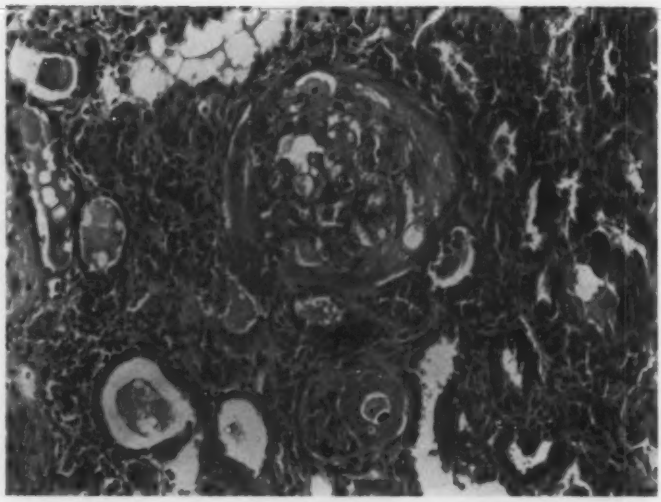

Fig. 6.—Fibrinoid necrosis of glomerulus and adjacent arteriole. Two months after renal ablation with 1% sodium chloride as drinking fluid. Hematoxylin-eosin stain; × 140.

tween adjacent tufts and between tufts and Bowman's capsule. Occasionally there was swelling and slight proliferation of epithelium, but well-developed crescents were unusual. Fibrinoid necrosis of tufts was fairly common (Fig. 6). A frequent lesion consisted of accumulation of fat in the form of large globules, which seemed to be mainly in extracapillary location. There was progressive hyalinization and eventual complete fibrous obliteration of glomeruli.

Another early lesion, focal in nature, involved proliferation of the cells lining the proximal convoluted tubules. The cells contained large vesicular nuclei, and the cytoplasm was reduced in amount and basophilic. The significance of this change is not clear.

Increased permeability of capillary tufts led to formation of colloid casts within the tubules, and this was sometimes marked. Otherwise there was tubular atrophy, associated with interstitial fibrosis and lympho-

Fig. 7.—Myocardial necrosis in rat drinking 1% sodium chloride. Two weeks after three-fourths renal ablation. Hematoxylin-eosin stain; × 120.

cytic exudate, probably secondary to the glomerular disease. Pus casts were observed within occasional tubules, and in addition there was rather infrequent pyelitis. However, the evolution and nature of the renal lesion did not suggest a pyelonephritic origin.

The remnant of kidney also showed intrarenal vascular disease involving small arteries and arterioles. The changes here were principally necrotizing, i.e., fibrinoid, rather than polyarteritic. As a rule the vascular disease seemed insufficient in extent to account for the diffuse glomerular destruction which was characteristic of the advanced renal lesion.

Cardiac Lesions.—These lesions were focal or widespread, varied from subendocardial to intramural or transmural in location, and involved either or both ventricles. On occasion lesions could be detected grossly as gray or grayish-white nodules. Microscopically, the muscle fibers revealed patchy degeneration and/or necrosis, sometimes associated with fibrinoid necrosis of vessels and also an interstitial fibroblastic reaction (Fig. 7). In older rats there were healed lesions consisting of fairly dense connective tissue.

Comment

The frequency with which hypertension follows reduction in renal mass depends on the amount of kidney that is removed. Thus, the incidence is rather low after unilateral nephrectomy, i.e., about 18%,[3-5] and substantially higher when more than one kidney is resected. From our results and those of others, high blood pressure occurs in from 45% to 69% of rats with from two-thirds to four-fifths renal ablation.[1-3]

In our study the time of onset of hypertension after three-fourths renal ablation was variable. The elevation in pressure was often slow to develop, requiring a period of weeks or months. At times the hypertensive levels were only moderate and not well sustained. Some animals were apparently refractory and failed to become hypertensive at all.

While the response of rats to removal of three-fourths of their renal substance was partly unpredictable, it became fairly uniform when 1% sodium chloride replaced water as the drinking fluid. Hypertension then developed much more rapidly; the level was higher and more sustained, and virtually all animals were affected. Moreover, salt accelerated and intensified the development of vascular and renal lesions. Such lesions occurred earlier in these rats than in the animals on water, were more diffuse, and progressed more rapidly. In this respect the picture in rats drinking 1% sodium chloride after three-fourths renal ablation tended to simulate human malignant hypertension.

Salt can produce hypertension in the rat without any reduction in renal mass.[6-10] In our laboratory, for example, 66% of rats with intact kidneys which drank 1% sodium chloride instead of water developed high blood pressure.[10] However, a period of about six to eight months on this regimen was usually required before hypertension occurred. Progressive reduction in kidney substance enhances the effect of salt; i.e., hypertension develops with greater frequency and in a shorter time.[5] Thus, 94% of unilaterally nephrectomized animals which drank 1% saline became hypertensive, usually in a few months. In the present study all rats placed on salt after three-fourths renal ablation were hypertensive within two weeks.

The fate of the kidney stump following subtotal renal ablation has been studied by several investigators.[2,11,12] It was found that when three-quarters of the kidney mass was removed, the remaining one-quarter first underwent compensatory hypertrophy and then developed progressive disease. The latter was attributed to the excretory overload placed on the small number of remaining nephrons. The glomeruli were especially vulnerable and were gradually destroyed. Since an increase in dietary protein accelerated the lesions, even with one-half ablation,[13,14] it was thought that excretion of end-products of protein metabolism, especi-

ally amino acids, constituted the injurious mechanism.[11,13-16]

Presumably because glomerular involvement was so pronounced, some authors designated the lesion in the renal stump as nephritis or glomerulonephritis.[12,14,17] However, it is doubtful whether such terms are applicable or desirable from the pathogenic standpoint. No evidence is available that the glomerular alterations arise on an infectious, including a pyelonephritic, basis or represent an immunoallergic process. A more appropriate term for the renal process would appear to be nephrosclerosis,[18,19] which in the rat comprises glomerular, as well as vascular, tubular, and interstitial changes.

Morphologically the disease in the kidney stump following subtotal ablation is essentially the same as that which develops in the kidneys of rats with salt,[5,20] steroid,[18] or renal hypertension.[19,20] In other words, hypertensive rats tend to develop a uniform type of renal lesion irrespective of the manner in which high blood pressure is induced. The lesion is not necessarily dependent on prior reduction in renal mass, since it can be produced by salt in rats with intact kidneys.[5,9] All the above observations also apply to the peripheral vascular disease associated with renal ablation hypertension.

The development of nephrosclerosis in the kidney stump of rats with three-fourths ablation was significantly hastened by salt loading. The lesion was often well advanced within two months, as compared with a six- or eight-month period for ablation rats on water. Like protein, salt is especially injurious to the kidney when the number of nephrons available for function is substantially reduced. The principal damage is to the glomeruli, especially the basement membranes of the capillary tufts. This may represent a direct toxic effect of salt on these structures, since the injury is acquired from handling or excreting excess sodium chloride.

Nephrosclerosis in the rat appears to stem primarily from destruction of glomeruli rather than from intrarenal vascular disease. However the latter probably plays a role also. Disease of the small arteries and arterioles was observed frequently in our animals as part of the developing nephrosclerotic process in the kidney stump. The lesions seemed to arise more or less simultaneously with those in other peripheral vessels and then roughly paralleled the latter in progression and severity. The vascular disease in the kidney was regarded not as a separate or distinct entity but as the renal component of widespread vascular disease. The changes in the vessels were likely to be absent or slight early in the course of hypertension and then to progress as the high blood pressure became chronic. It appears likely that the similarity between the renal vascular lesions and those elsewhere in the body in respect to morphology, time of onset, and development can be extended to include the mode of origin.

An important consideration in the present study is the pathogenesis of renal ablation hypertension. The high blood pressure in our animals was based on reduction in kidney mass, and hence the problem is how such reduction caused, or contributed to, the origin of hypertension.

According to one theory, the kidneys normally secrete a substance which is antihypertensive and serves to maintain normal blood pressure.[21] Loss of such a substance through reduction in kidney mass would tend to yield hypertension. This is the mechanism which is proposed to explain so-called renoprival hypertension.

This concept poses some questions in reference to subtotal renal ablation. It does not apply uniformly to rats with three-fourths ablation; i.e., some of these may become hypertensive fairly rapidly, others only after a lapse of weeks or months, and some not at all, even after an interval of 10 months. However, variation in the amount of antihypertensive substance produced by the remaining quarter of kidney might explain these irregularities. How the theory would relate to the conversion of

normotensive rats with three-fourths ablation to animals with acute fulminant hypertension when 1% sodium chloride replaces water for drinking is obscure. Here salt appears to act as a potent hypertensive agent conditioned by reduction in renal mass. Perhaps the salt in some way suppresses the production and/or activity of antihypertensive material derived from the kidney stump. Otherwise the alternative is that salt acts to elevate blood pressure in an independent manner.

Another mechanism for experimental hypertension, especially in reference to renal injury, is based on elaboration of a hormonal pressor agent by the kidney.[22] This may not be applicable to renal ablation hypertension, since the kidney stump initially suffers no intrinsic damage, nor is it clear that there is impairment of its blood flow. The suggestion has been made that the hypertension following renal ablation results from prior development of organic disease in the stump.[23] Our study yielded evidence to the contrary. Interval killing of animals with three-fourths ablation showed that at the onset of hypertension lesions of the glomeruli and of the renal arterial tree were either minimal or absent. On the other hand, there was sometimes a noteworthy dissociation between nephrosclerosis and hypertension among the rats subjected to three-fourths ablation which drank water. A number of these rats were normotensive several months after nephrectomy and yet had well-developed nephrosclerosis. Hence the latter per se by no means guarantees the occurrence of high blood pressure.

Another mode of origin is possible for the hypertension which develops in rats subjected to three-fourths renal ablation and then maintained on standard diet.[5] This possibility is based on the observation that the hypertensive response of the rat to salt increases as kidney mass is further reduced. It would seem plausible that some rats which are left with only one-fourth of their kidneys might be sensitive to average amounts of exogenous salt, such as are present in a standard diet, and that in due time this could suffice to produce high blood pressure, as well as vascular and renal disease without the need for salt loading. Such a view implies that renal ablation hypertension may be a form of salt hypertension.

The mechanism by which salt acts to produce hypertension in the rat is still not clearly established. It may be through retention of salt and water in the tissues, thereby giving rise to altered composition and distribution of electrolytes and water within the peripheral vascular bed, which could lead to enhanced tonus of the vessels. Preliminary data on the mesenteric arteries, while not conclusive, provide suggestive support for this concept.[24] While it would appear that the kidney need not play a primary role in the origin of salt hypertension, it probably has an important ancillary one which is related to its capacity for salt excretion. This would apply especially to rats with three-fourths renal ablation, since significant reduction in renal mass may serve to augment the retention of sodium and water in the tissues.

Summary

Most rats maintained on standard diet after removal of three-fourths of their kidney mass become hypertensive. This is associated with the development of diffuse vascular disease, cardiac hypertrophy, and nephrosclerosis of the kidney stump. All these changes follow the high blood pressure rather than preceding it. Both the vascular and renal lesions are essentially similar to those observed in rats rendered hypertensive by means of salt, steroids, or renal injury.

Salt is highly injurious when administered to animals with three-fourths renal ablation. It intensifies the hypertensive vascular disease and accelerates the development of nephrosclerosis. The action of salt may be independent of the elaboration of hormonal agents by the renal stump. It is suggested that renal ablation hypertension has the same mode of origin as salt hy-

pertension and is related to disturbances in electrolyte and water metabolism.

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Intracranial Sarcomas Produced by Polyoma Virus in Syrian Hamsters

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The development of pleomorphic tumors of the parotid gland in mice inoculated on the day of birth with cell-free filtrates of murine leukemic tissues has been described by Gross [1] and by Stewart [2,3] and has been confirmed in several other laboratories.[4-6] Subsequently, it has been shown that the cell-free filtrates which produced these tumors contained a virus, the polyoma virus, which could be grown in tissue culture and which produced a cytopathogenic effect in cultures of minced mouse embryo.[7,8] In addition to parotid gland tumors, mice inoculated on the day of birth with preparations of the virus grown in tissue culture have developed tumors in other salivary glands, the thymus, the adrenal glands, the mammary glands, and other sites.[7]

Eddy and her associates found that the virus agglutinated erthyrocytes from the guinea pig, hamster, human, and several other species.[9] Purification of virus in tissue culture fluids by adsorption and elution from red blood cells and by density-gradient centrifugation has been possible; and when material prepared in this manner was examined with the electron microscope, characteristic particles with a diameter of approximately 44 mμ were observed.[10]

Newborn and young Syrian hamsters inoculated with polyoma virus have developed multiple sarcomatous and angiomatous tumors in various sites.[11] Tumors have also been produced in rats and rabbits when newborn animals have been inoculated with preparations of the agent.[12,13]

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In the reported studies of polyoma virus infection in Syrian hamsters, the virus has been inoculated subcutaneously, and sarcomatous lesions have been produced in the heart, kidneys, subcutaneous tissues, and gastrointestinal tract.[11] Angiomatous lesions have been produced in the liver, ovaries, and lungs, and proliferation of vascular endothelium has been prominent in some of these.[11] No gliomas have been observed in the central nervous system, although Stanton has seen sarcomatous lesions of the meninges in two animals inoculated subcutaneously with polyoma virus.[14]

In studies of experimentally produced brain tumors in laboratory animals, direct implantation into the brain, or inoculation of the brain, with carcinogenic agents has been generally employed. The experimental production of brain tumors in mice by the intracerebral implantation of pellets of carcinogenic hydrocarbons has been extensively studied, and various types of mesenchymal tumors and gliomas have been described.[15-21] Rous sarcoma virus has been injected intracerebrally in young chickens with production of infiltrating sarcomatous lesions of the meninges and brain.[22,23] We therefore thought that it would be of interest to study the response of the brain of a laboratory animal susceptible to infection with polyoma virus after intracerebral inoculation of the agent.

Materials and Methods

Virus.—The strain of polyoma virus used in these studies was originally isolated from a cell-free filtrate of an extract of a parotid gland tumor of a C3H/Bi. mouse. The details of the derivation of this strain of virus have been given by Dawe and his associates.[24] The virus has been grown in cultures of milk-adapted murine

lymphoma cells (Strain P388D1) according to methods previously described.[26] In the intracerebral studies, a pool of 10th-passage material was used. The virus pool was prepared by inoculating 2 oz. prescription bottle cultures of milk-adapted P388D1 cells with 9th-passage virus and observing them until severe cytopathogenic effect was evident. The cultures were then frozen and thawed and the fluids pooled and stored in 2 ml. aliquots in rubber-stoppered tubes at —20 C. Virus preparations were thawed immediately before injection into animals. A sample of this pool was titrated by serial tenfold dilutions in milk-adapted P388D1 cells and was found to contain $10^{4.6}$ $TCID_{50}$ (tissue culture infectious doses) per 0.25 ml.

Hamsters.—Pregnant Syrian hamsters (Cricetus auratus) were obtained from the animal production unit of the National Institutes of Health. The litters from these were used for intracerebral inoculation. Two litters (23 animals) were inoculated when less than 24 hours old; one litter (4 animals) was inoculated at 8 days of age; one litter (9 animals), at 9 days of age, and one litter (8 animals) at 12 days of age. Two litters, 10 days of age (10 animals), were inoculated intracerebrally with control fluid. This consisted of medium from P388D1 cultures free of polyoma virus and included cellular debris, as well as fluid medium. The control material was carried through one cycle of freezing and thawing prior to use in animals.

Methods of Inoculation.—Tuberculin syringes with No. 27-gauge needles were used. The bone of the skull was readily pierced with the needle and 0.02 ml. of inoculum was injected into the midportion of the right cerebral hemisphere of the animals less than one day of age. The older animals received 0.10 ml. of inoculum in the midportion of the right cerebral hemisphere.

Autopsies.—Complete autopsies were performed on all animals except in cases in which cannibalism made this impossible. Tissues were fixed either in Zenker's solution with 10% formalin or in formol-mercury bichloride (Sublimate). As a rule, entire

Syrian Hamsters Inoculated Intracerebrally with Polyoma Virus

Animal No.	Age at Inoculation	Age at Autopsy	Outcome *	Virus Dose $TCID_{50}$ †	Lesions				
					Brain	Liver	Heart	Kidney	Lungs
1	1	30	F	2,600 $TCID_{50}$	Head eaten	+	—	+	+
2	1	31	F	"	+	+	—	+	—
3	1	37	F	"	+	+	+	+	—
4	1	37	F	"	+	—	+	+	—
5	1	40	F	"	+	—	+	—	—
6	1	40	F	"	+	+	—	+	—
7	1	41	F	"	+	+	—	+	—
8	1	46	F	"	—	+	+	—	—
9	1	46	F	"	Head eaten	+	—	—	—
10	1	62	F	"	—	+	—	—	—
11	1	70	K	"	—	+	—	—	+
12	1	70	K	"	—	—	—	—	—
13	1	70	K	"	+	+	—	—	—
14	1	70	K	"	—	+	—	—	—
15	8	48	F	13,000 $TCID_{50}$	Head eaten	—	—	—	—
16	8	53	K(M)	"	+	—	—	—	—
17	8	56	K(M)	"	+	—	—	—	—
18	8	84	K(M)	"	+	—	—	—	—
19	9	43	K(M)	"	+	+	—	—	—
20	9	54	K(M)	"	+	—	—	—	—
21	9	54	K(M)	"	+	—	—	—	—
22	9	64	K(M)	"	+	—	—	—	—
23	9	137	F	"	+	—	—	—	—
24	9	158	K	"	—	—	—	—	—
25	12	44	K	"	+	+	—	—	—
26	12	56	F	"	+	+	+	—	—
27	12	63	F	"	+	+	—	—	—
28	12	64	K(M)	"	+	—	+	—	—
29	12	65	K(M)	"	+	—	—	—	—
30	12	104	K	"	—	—	—	—	—
31	12	104	K	"	+	+	—	—	—
32	12	104	K	"	—	—	—	—	—

* F=found dead; K(M)=killed when moribund; K=killed.
† Tissue culture infectious doses.

brains were removed and placed in fixative for two to four hours. They were then sectioned coronally or horizontally and again placed in the fixative for 20 to 24 hours. In animals in which there had been considerable autolysis, the brains were initially fixed in the cranium for about four hours and then removed for sectioning and further fixation. Sections for microscopic study were stained with hematoxylin and eosin, Mallory's azan method, Bielschowsky-Foot silver impregnation for reticulum, and phosphotungstic acid hematoxylin.

Results

Forty-four young hamsters from five litters were inoculated intracerebrally with polyoma virus, and 32 of these animals survived the immediate trauma of inoculation. Data, including age at time of inoculation, age at autopsy, and significant pathologic findings in these 32 hamsters, are presented in the Table.

No immediate paralysis or other sign of central nervous system damage was noted in the animals that survived the inoculation trauma. About one month after inoculation, some of the animals were observed to be lethargic and to have paralysis of the lower extremities. The lethargy progressed to coma and finally to death. Some animals, however, in which no preceding signs of disease were noted, were found dead. As shown in the Table, 14 of the 32 animals were found dead in their cages and in 3 of these the heads had been eaten by the cage mates so that examination of the brain was not possible. Of the remaining 11 animals found dead, intracranial sarcomatous lesions produced by polyoma virus were found in 9. To avoid autolysis and cannibalism, nine animals were killed when moribund. Intracranial sarcomas were found in all of these. Nine animals were killed at times when they had no objective evidence of intracranial disease, and sarcomas were found in the brains of three of these.

Of the 10 hamsters inoculated with control fluid, 8 survived the inoculation. None showed signs of intracranial lesions, and all were killed 74 days after inoculation. Complete autopsies were performed of all of these, and no lesions were found.

Fig. 1.—Brain of hamster inoculated intracerebrally with polyoma virus. There is a large tumor in the parietotemporal region of the right cerebral hemisphere with compression of adjacent structures and distortion of the ventricular system.

Pathology of Intracranial Tumors.—Grossly, when the calvaria was removed, the cerebral hemisphere on the side of inoculation was usually enlarged. On section, nodular areas of gray-white or tan tumor tissue were seen within the cerebral substance, with compression and distortion of the ventricular system and adjacent structures of the brain (Fig. 1). In some cases, a single nodule or multiple nodules of tumor involving the meninges could be seen on the dorsal surface of the brain. On section, it was evident that some of the meningeal nodules compressed underlying brain with little infiltration (Fig. 2), while others infiltrated underlying parenchyma more extensively (Fig. 3). In one of the animals that survived for 137 days after virus inoculation, a large tumor of the meninges was found in the frontal region with compression of the brain posteriorly.

Histologically, the intracranial tumors were sarcomas (Figs. 4 and 5) and were essentially similar to the sarcomatous tumors of the kidneys, heart, and subcutaneous tissues described by Eddy and her associates.[11] In some areas the tumor cells were arranged in interlacing bundles, while in other areas they had a perivascular arrangement around small blood vessels within the tumor. The tumor cells were elongated with hyperchromatic pleomorphic nuclei and small amounts of poorly defined, faintly basophilic cytoplasm. Clear areas

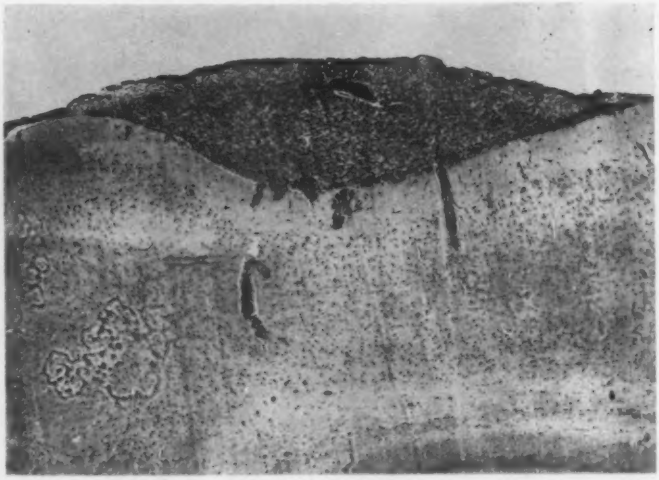

Fig. 2.—Section of meningeal tumor arising in hamster inoculated intracerebrally with polyoma virus. The sarcomatous tumor in the meninges is well demarcated from underlying brain, although there is some tumor around blood vessels extending into the cerebral tissue. Hematoxylin and eosin; reduced to 80% of mag. × 60.

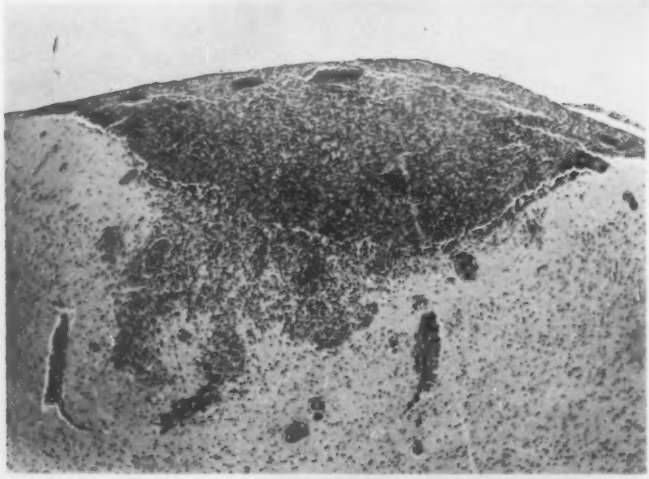

Fig. 3.—Section of sarcomatous tumor of meninges arising in hamster inoculated intracerebrally with polyoma virus. There is extension of tumor from meninges into underlying cerebral tissue. Hematoxylin and eosin; reduced to 80% of mag. × 60.

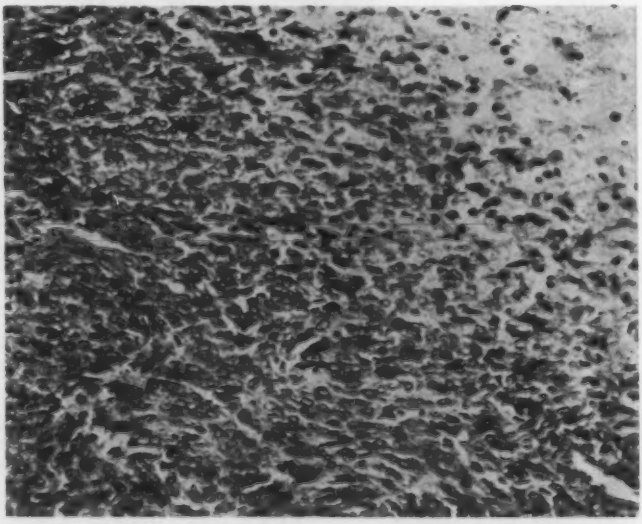

Fig. 4.—Section of sarcoma in the brain of a hamster inoculated intracerebrally with polyoma virus. The tumor cells are elongated and arranged in bundles. Infiltration of adjacent cerebral tissue can be seen. Hematoxylin and eosin; reduced about 80% of mag. × 270.

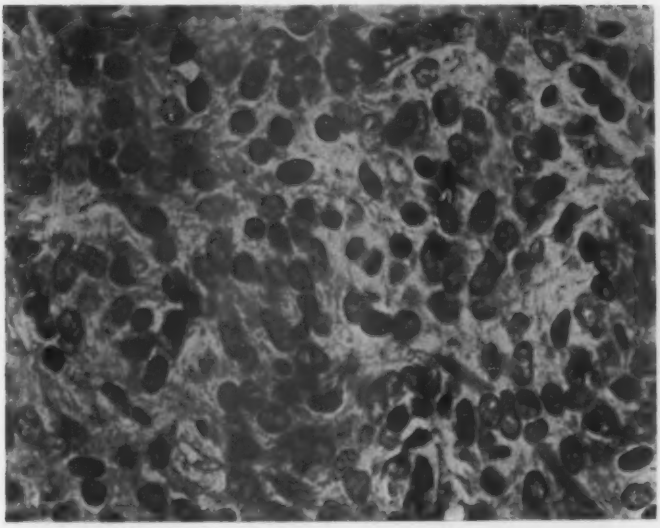

Fig. 5.—Section of sarcoma in the brain of a hamster inoculated intracerebrally with polyoma virus. A bipolar mitotic figure is seen in the center of the photograph. Some of the tumor cell nuclei have central clear areas, with margination of clumped chromatin along the nuclear membrane. Hematoxylin and eosin; reduced to about 80% of mag. × 880.

were seen in the nuclei of some of the cells, with margination of clumped chromatin along the nuclear borders. No intranuclear or intracytoplasmic inclusion bodies were identified. Mitoses were numerous in some areas and were usually bipolar, although some atypical figures were seen. Large areas of necrosis were rare, although the nuclei in some of the tumor cells showed pyknosis and karyorrhexis. Large numbers of plasma cells were present within some of the tumors.

At the margins of the tumors, concentric collections of tumor cells surrounded small cerebral vessels, filling the perivascular spaces (Fig. 6). These may represent extension of the tumor cells along the perivascular spaces, a feature commonly seen in intracerebral tumors in man; however, in the case of polyoma virus tumors, it also

Fig. 6.—Section of sarcoma in the brain of a hamster inoculated intracerebrally with polyoma virus. At the margin of the tumor, the perivascular spaces are filled with tumor cells concentrically arranged around the vessels. Hematoxylin and eosin; reduced to about 80% of mag. × 270.

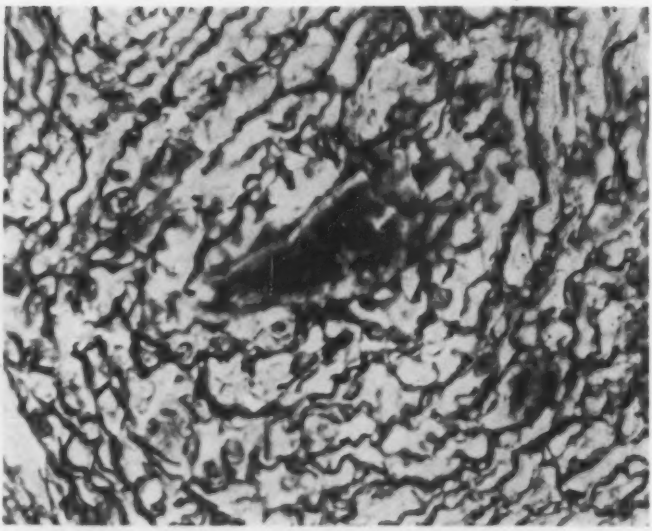

Fig. 7.—Section of sarcoma in the brain of a hamster inoculated intracerebrally with polyoma virus. The silver impregnation preparation shows large amounts of reticulum in the tumor. The perivascular arrangement of the tumor cells is also well demonstrated. Bielschowsky-Foot silver impregnation method; reduced to about 80% of mag. × 880.

seems likely that some of these lesions are the result of spread of virus along the spaces with production of tumor cells in the perivascular areas by the virus.

In the sections stained by Mallory's azan method, collagen was demonstrated in the tumors. Many reticulum fibers could be seen in the Bielschowsky-Foot silver impregnation preparations, and the perivascular arrangement of the tumor cells was well demonstrated with this technique (Fig. 7). No glial fibers were found in the tumors in sections stained by the phosphotungstic acid-hematoxylin method. All of these findings support the opinion that these tumors are sarcomas derived from connective tissue, and not polar spongioblastomas or piloid astrocytomas.

Visceral Lesions.—The frequency of lesions in the viscera is indicated in the Table. The histopathology of the sarcomatous lesions of the heart and kidneys and of the

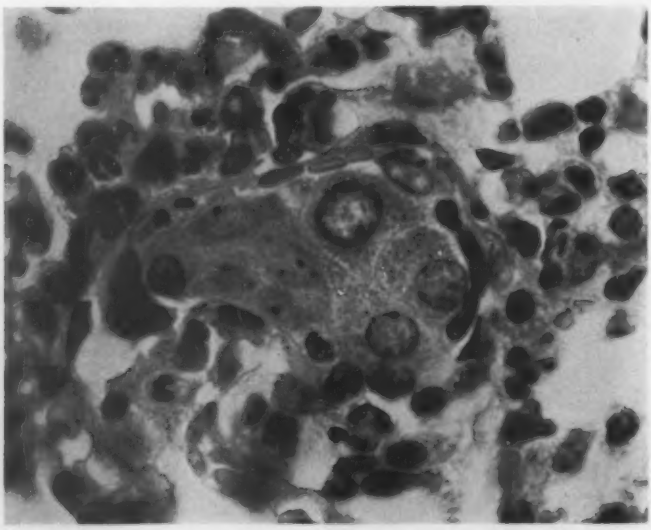

Fig. 8.—Section of lung of hamster inoculated intracerebrally with polyoma virus. A group of liver cells is seen within the lumen of a capillary in an alveolar septum. The animal had a large angiomatous tumor of the liver, and multiple liver-cell emboli, apparently originating from this vascular liver tumor, were found in the capillaries of the lungs. Hematoxylin and eosin; reduced to about 80% of mag. ×1,260.

vascular tumors of the liver was essentially similar to that previously described.[11] Of the 32 animals, 6 had sarcomatous lesions of the heart; 4 of these 6 had been inoculated on the day of birth. Six of the thirty-two had sarcomatous lesions of the kidneys; all of these were in the group inoculated on the day of birth. Of the 32 animals, 16 had liver tumors, and 11 of these were in the group inoculated on the day of birth.

In addition to the findings listed in the Table, one of the animals inoculated on the day of birth (Hamster 3) had a sarcomatous lesion in the wall of the esophagus and a similar lesion adjacent to one of the parotid glands. Two of the animals that had vascular tumors of the liver (Hamsters 13 and 14) also had emboli of liver cells in capillaries of the alveolar septae of the lungs (Fig. 8).

Comment

Malignant mesenchymal tumors originating in the central nervous system are rare in man, and they are thought to arise from the connective tissue of the pia mater on the surface of the brain and in the sulci, and from the adventitia of meningeal and cerebral blood vessels.[26,27] Grossly, these human tumors are firm gray masses, which usually appear to be sharply circumscribed from surrounding brain. Occasionally, they seem to be multicentric, with a number of discrete nodules found in different parts of the brain. Microscopically, the tumor cells are spindle shaped and may be arranged in interlacing bands. They infiltrate the leptomeninges and may extend along the perivascular spaces into the neural parenchyma. Collagen and reticulum fibers can be demonstrated with appropriate special staining methods. Zimmerman and his associates[27] have referred to these tumors in man as "leptomeningeal sarcomas," and they are similar in many gross and microscopic features to the intracranial tumors produced by polyoma virus in Syrian hamsters. It should also be noted that both the leptomeningeal sarcomas of man and the intracranial sarcomas produced by polyoma virus in the hamster resemble the intracranial lesions of the young chicken produced by intracerebral inoculation of Rous sarcoma virus.[22,23] These similarities suggest the desirability of attempting to isolate a virus from cases of leptomeningeal sarcoma in man.

According to our observations, the histogenesis of the intracranial polyoma virus tumor in the hamster is similar to the histogenesis proposed by Zimmerman and his associates for leptomeningeal sarcoma in man.[27] Some of the hamster sarcomas appear to arise from connective tissue in the pia mater on the surface of the brain and in the sulci. In others, the tumor cells have a perivascular arrangement and appear to arise from the adventitia of small meningeal and cerebral vessels. It is of interest that, although gliomas have been produced in experimental animals after intracerebral implantation of carcinogenic hydrocarbons, no tumors of glial origin have as yet been observed in animals inoculated intracerebrally with either polyoma virus or the Rous sarcoma virus. This may be of some importance in selection of tumor material from man for virus isolation studies.

Tumors in the heart, liver, kidneys, and lungs are commoner in the hamsters inoculated intracerebrally on the day of birth than in the animals inoculated at 8, 9, and 12 days of age. This may be a result of greater susceptibility of the newborn to carcinogenic exposure of any type,[28] as well as, possibly, to a greater tendency for virus to disseminate hematogenously in the very young animals. The latter possibility might be established experimentally by attempting to isolate polyoma virus from the blood after intracerebral inoculation in animals of varying ages.

The availability of quantitative virological and immunological methods, such as infectivity titration in tissue culture,[8,29] hemagglutination,[9] and complement fixation[30] for the study of polyoma virus, should make it possible to obtain precise data on the relationship of the virus to the intra-

cranial sarcomas in the hamsters. Such quantitative studies have already been undertaken with Rous sarcoma virus inoculated intracerebrally in young chickens, and data concerning growth of virus within the brain [31,32] and the relationship of amount of virus in the inoculum to the survival time of the animals and the virus content of the tumors [23] are available. A comparison of the findings in quantitative studies of polyoma virus in hamster brains with similar data obtained from the avian tumor virus system might be of interest.

Summary

Intracranial sarcomas have been produced in Syrian hamsters inoculated intracerebrally with polyoma virus. These mesenchymal tumors apparently arise from connective tissue in the pia mater on the surface of the brain and in the sulci, and from the adventitia of small meningeal and cerebral vessels. The tumors have developed in animals inoculated on the day of birth and at 8, 9, and 12 days of age, and they have been found in animals which have died as soon as 31 days after inoculation. Histologically, the intracranial sarcomas of the hamster produced by polyoma virus resemble the sarcomatous lesions of the brain produced in the young chicken by intracerebrally inoculated Rous sarcoma virus. The hamster tumors also resemble the rare mesenchymal tumors of the brain in man which have been called leptomeningeal sarcomas. These observations suggest that attempts to isolate oncogenic viruses from human leptomeningeal sarcomas might be successful.

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An Unusual Form of a Neurovascular Hamartoma

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Though its manifestations are protean and associated lesions manifold,[5,6,7,22] neurofibromatosis—essentially an inherited, dysontogenetic hamartomatous malady—discloses itself commonly as a disturbance of the peripheral nerves and of the autonomic nervous system whereby solitary or multiple tumefactions arise which characteristically are accompanied by pedunculated or mollusc-like cutaneous neural swellings. Often the proliferating nerve-sheath tissue intermingles profusely and merges imperceptibly with the surrounding tissue.

Anomalies of structures other than nerve sheath happen so frequently in neurofibromatosis that some authorities[5-7] have suggested that the neural lesions represent only a one-sided manifestation of a basic systemic upset. Areas of cutaneous pigmentation, varying in intensity, are common, and true melanomata, including malignant variants, sometimes may be present, occurring either as independent and associated or as composite lesions, mingling with an underlying neurofibromatoid proliferation. Some such nevi contain angiomatous as well as neural elements, both components being intimately and confusingly mixed together. Not only may cutaneous angiomata accompany neurofibromatosis, as independent malformations, but the neural lesions may be either profusely vascular or composed of a mixture of angiomatous and neurofibromatoid tissue so closely interwoven as to suggest that the vascular proliferation was induced by the Schwannian proliferation.

The association of glomus-like structures and glomus-cell proliferation with neurofibromatous tissue, however, though possible, as in the case described below, is obviously extremely rare, as no example can be traced in the literature. Normal glomera have a complex architecture[8,10,17]; they are richly supplied by nerves, often arranged in a plexiform manner. In the case described below it is believed that the neurofibromatoid process affected the nerves of glomera, and also those supplying the blood vessels in the regions involved by neurofibromatosis.

Of interest, too, in this case, is the differentiation of organoid structures of neuroectodermal derivation—Verocay bodies, *lames foliacées*, or Meissneroid complexes—as intrinsic components of the neurofibromatoid tissue.

Report of a Case

History

A Negro woman 28 years of age arrived at the emergency room of the University of Chicago Clinics at 5 a.m. on Nov. 30, 1958, complaining of a rapidly enlarging swelling on the left side of her neck. She had noted a "pins and needles" sensation in the posterior triangle of her left neck at 8 p.m. the previous day; she rubbed this area, and soon after a painless swelling appeared.

The past history revealed a fracture of the left clavicle at the age of 3 years, three spontaneous abortions in the second trimester of pregnancy, and a stillbirth in 1948.

Examination of records at another hospital shortly after the death of the patient revealed a hospitalization from March 3 to March 16, 1951, for an occasionally painful and tender mass in the neck. This mass had been present for approximately two years, and she had experienced several dizzy spells during the same period. A soft, compressible, "spongy, lemon-sized" mass was found in the left side of the neck, increasing in size on lying down or straining. Pressure on the mass did not affect systemic arterial blood pressure. Aspiration of the mass returned venous blood. Surgical exploration of the neck revealed stigmata (fibrous replacement of the medial half

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of the clavicle and retractive scarification of the detached left sternocleidomastoid muscle) of the old clavicular injury, which was probably due to a local defect of the clavicle related to the neurofibromatous lesions and varicosities of the external jugular vein. The varicosities were ligated and the left platysma coli and sternocleidomastoid muscles were biopsied. The specimen contained multiple neurofibromata with Verocay bodies. A large postoperative hematoma required surgical evacuation, and she was discharged two days later.

On admission, the patient was dyspneic, and a nonpulsating mass was present in the entire left side of the neck, extending beyond the midline posteriorly and anteriorly. The overlying skin was tense, and no bruit was audible; a 3×6 cm., deeply pigmented brown-blue area was noted in the center of the left posterior triangle, which the patient claimed had been there all her life. Blood pressure was 96/65, pulse 65 a minute, hemoglobin 9.6 gm. %, hematocrit 24%, WBC 7,500 per cubic millimeter. The swelling was needled and blood recovered. Immediate surgical exploration was undertaken. Approximately 500 cc. of clotted blood was evacuated from both anterior and posterior left cervical triangles. A longitudinal gap was observed in the wall of the collapsed left internal jugular vein, and minimal manipulation resulted in fragmentation of the vessel. Oozing from the tissues was not controllable by intravenous ephedrine. As the bleeding started high in the neck, the common carotid artery was clamped and ligated. Both clamp and subsequent ligatures cut through the proximal segment of the carotid artery. Sternotomy was performed, and no sooner was the carotid artery bleeding controlled at the vessel origin than the subclavian artery began to bleed. The subclavian and innominate veins then bled and disintegrated, flooding the left thoracic cavity. Many ligatures were placed on these vessels and on the associated numerous, dilated, oozing venous channels. The neck was then packed and tracheotomy performed.

Postoperatively, blood pressure readings ranged between 50 and 90 systolic. She was aided by a respirator, but a steady ooze of blood was perceived in the nasopharynx. Antibiotics, calcium gluconate, protamine, and prednisolone were administered, and blood was drained from the left thorax. She was given 28 pints (14,000 cc.) of blood by transfusion.

The patient responded briefly to vocal stimuli that evening, but her blood pressure fell. By 9:30 a.m. the following morning her blood pressure could not be measured. Her urinary output during more than 24 hours of hospitalization had been 60.0 cc.

Autopsy (six hours after death)

The body, measuring 156 cm. in length and weighing 49 kg., was that of a well-nourished and well-developed young Negro woman. Rigor mortis was well developed in the muscles of the extremities and neck.

The operative wound site bulged above the adjacent skin surface. Posterior portions of the neck and head were edematous, and three rubber drains had been inserted in the chest cavities and mediastinum. Portions of the left sternocleidomastoid muscle and of the left clavicle were absent.

The left common carotid artery stump was ligated at the aorta; the distal branches were ligated above the bifurcation. Bloody material was present in the upper mediastinum under the surgically split and repaired sternum. Each pleural cavity contained 200 cc. of sanguineous fluid.

The intact heart weighed 236 gm. Fine atheromatous streaks were seen in the intima of the ascending and abdominal aorta and carotid arteries. The aortic circumference was reduced, measuring 35 mm. at the level of the diaphragm. The aortic wall was 1 mm. thick. The left cartoid artery stump had a 0.5 mm. wall. The left subclavian artery was tied off at its origin. The right lung weighed 580 gm., the left 470 gm., and large amounts of foamy fluid escaped from the hyperemic cut surfaces.

The peritoneal cavity was intact. Small mucosal hemorrhages were seen in the stomach, ileum, and rectum. The liver weighed 960 gm., and two well-circumscribed, yellow, firm nodules bulged from the cut surface of its caudate lobe. One measured 0.5 cm. and the other 1.6 cm. in diameter. A pigmented stone was present in the gallbladder. The pancreas weighed 89 gm. and appeared normal.

The right kidney weighed 71 gm., the left 76 gm. The surface of the left kidney was smooth, but many deep scars, 6-7 mm. in length, were present on the right. The endometrium was hemorrhagic. The spleen weighed 96 gm., and the lymph nodes were of normal size. The pituitary gland appeared somewhat swollen. The symmetrical thyroid gland weighed 8.5 gm.; each adrenal gland weighed 6.5 gm. The brain weighed 1,220 gm., and the pial vessels were engorged.

Microscopic Features

The skin from the pigmented area on the left side of the neck showed an attenuated, but deeply pigmented epidermis and collections of melanophores in the neurofibromatoid tissue, which had replaced the dermis (Fig. 1) and extended into the subcutaneous tissue (Figs. 2 and 3). In its superficial portions, the neurofibromatous proliferation consisted of sheet-like areas and small

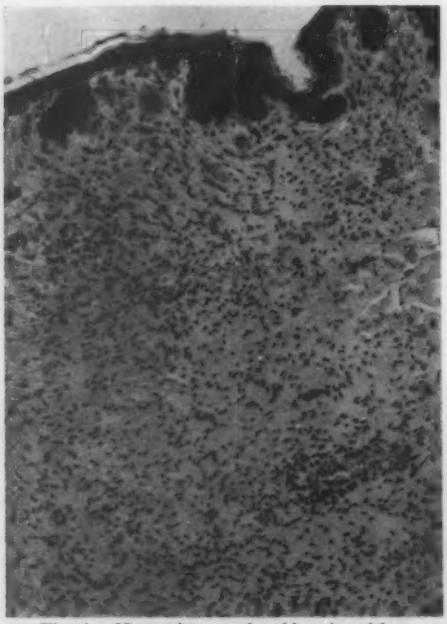
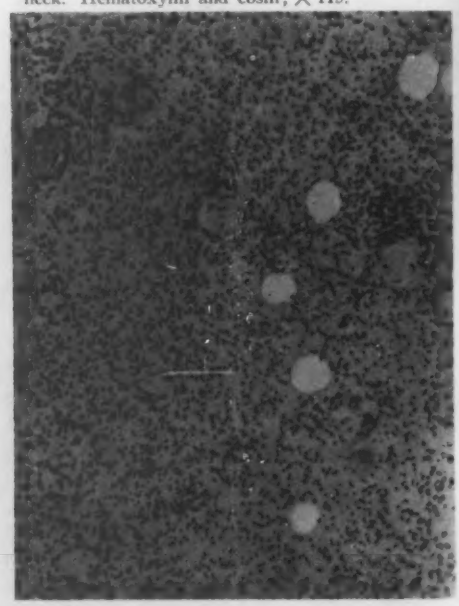


Fig. 1.—Hyperpigmented epidermis with underlying diffuse neurofibromatous tissue. Region of old birthmark, left side of neck. Hematoxylin-eosin stain; × 105.

Fig. 2.—Dermal and subcutaneous component showing fat and some neurilemmal differentiation. Region of old birthmark, left side of neck. Hematoxylin and eosin; × 115.



bundles of spindle cells with vesicular nuclei and pale cytoplasm and poorly developed intercellular stroma collagen; in the deeper portions there were many Verocay bodies *(lames foliacées)*, surrounded by compactly arranged Schwann cells (Fig. 3). Such structures were found among the neurofibromatoid tissue in many other regions (Fig. 4). Similarly, the left sternocleidomastoid muscle at the time of surgery in 1958 and in 1951 showed numerous Verocay bodies and compact proliferation of Schwann cells (Fig. 14).

Fig. 3.—More obvious neurilemmal differentiation (Verocay bodies) in subcutaneous portion of lesion. Region of old birthmark, left side of neck. Hematoxylin and eosin; × 120.

Except for the neurofibromatous swelling affecting the vagus nerve (Fig. 11), not only fat but also angiomatous tissue formed an integral component of the lesions. In addition, there were many arteriovenous anastomoses, including some well-formed glomera (Fig. 9), and numerous vessels were surrounded by a cuff of glomus cells (Figs. 8 and 10).

Such a neurofibromatous tissue extended to involve intimately both large and small

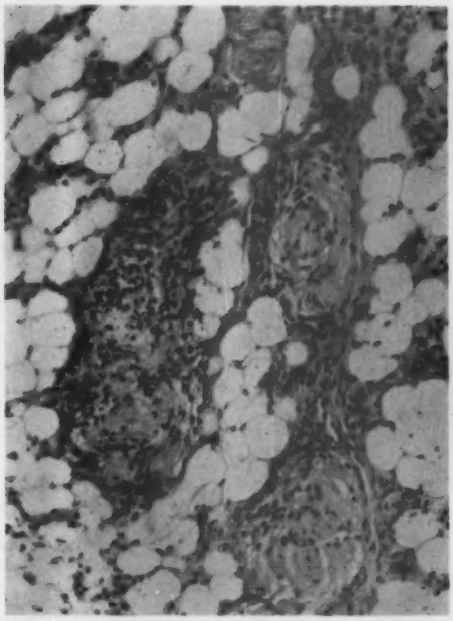


Fig. 4.—Neurilemmal differentiation and fat. Region of left supraclavicular plexus. Hematoxylin and eosin; × 140.

blood vessels (Figs. 5 and 6). The intima of the left carotid artery was focally thickened by relatively cellular fibroid tissue, in some places composing almost one-third of the vessel wall (Fig. 13); the media showed a marked scleral change. The neurofibromatous proliferation was related to the disruption and blurring of the media-ad-

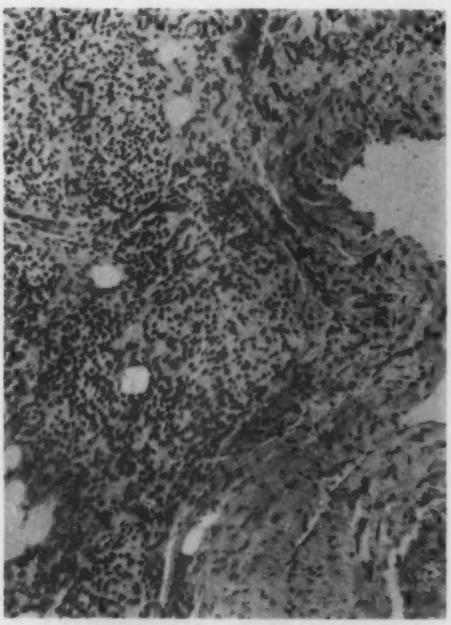


Fig. 5.—Blurring and involvement of adventitia of vessel by neurofibromatous tissue. Media shows sclerotic changes. Region of left supraclavicular plexus. Hematoxylin and eosin; × 120.

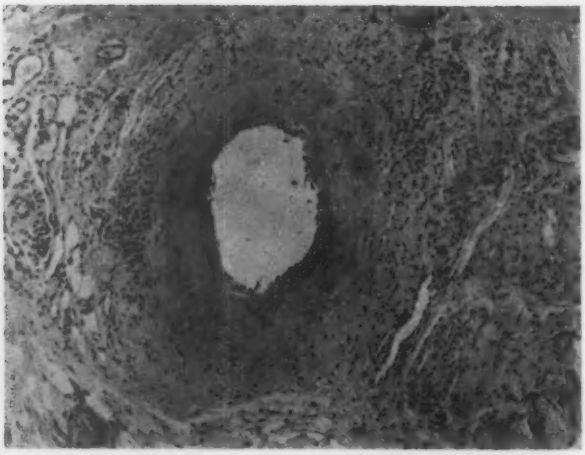


Fig. 6.—Extension of neurofibromatous tissue into vessel wall showing medial sclerosis. Region of convexity of aortic arch and pulmonary artery. Hematoxylin and eosin; × 80.

ventitial border and the fibrillary and hyaline changes in the inner media and intima. Medial degeneration of the right carotid artery was minimal, but the adventitia of the thoracic aorta contained neurofibromatoid tissue, similar to that found in the left neck region, and medial degeneration was pronounced. The upper abdominal portion of the aorta was also involved by neurofibromatosis, and so was the trachea at its bifurcation (Fig. 7) and the adjacent pulmonary artery, which, in addition, was affected by medial sclerosis. Thrombi and emboli were present in the

Fig. 7.—Neurofibromatous tissue with large vascular channels under cartilage of main bronchus near tracheal bifurcation. Hematoxylin and eosin; × 60.

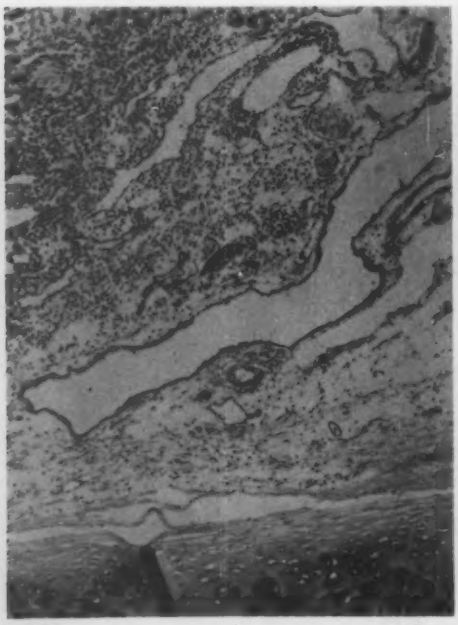


Fig. 8.—Vessel surrounded by collar of pericytes near area of neurilemmal differentiation. Region of left carotid sheath and nervus vagus. Hematoxylin and eosin; × 165.

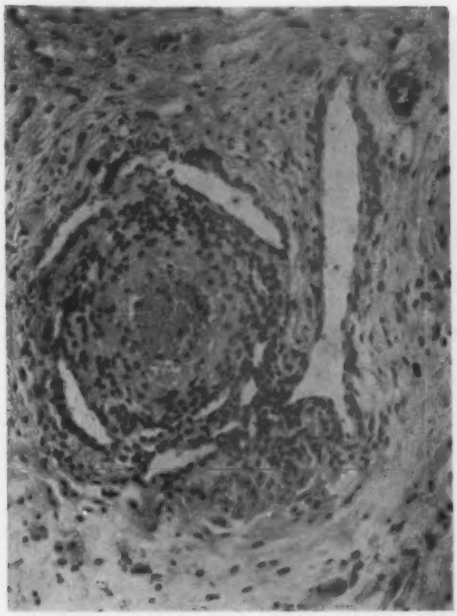


Fig. 9.—Glomus-like arteriovenous shunt. Region of left carotid sheath and nervus vagus. Hematoxylin and eosin; × 165.

small pulmonary arteries; the connective tissue surrounding these vessels was disposed in condensed concentric, eosinophilic lamellae. Pulmonary edema and congestion were evident, and some alveoli contained hemorrhagic material.

The two circumscribed, but nonencapsulated nodules found in the liver were zones of adenomatous development containing foci of bile-duct hyperplasia associated with anomalous blood vessels (Fig. 12), some of which were surrounded by thin cuffs of glomus cells and arteriovenous shunts. Around these foci the liver-cell cords were arranged in large, irregular lobules.

Marked lobular hyperplasia was found in the breasts. The myometrial vessels were dilated; the endometrium was congested. Hemorrhages were found in the mucosa of the colon and ileum. A small fibrocaseous

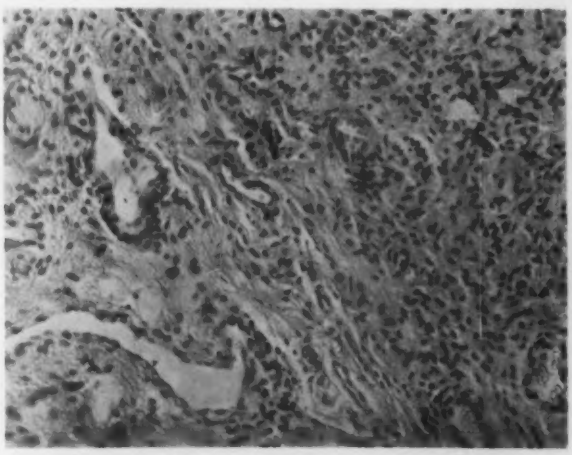


Fig. 10.—Vascular area, some vessels surrounded by thin band of pericytes. Region of left carotid sheath and nervus vagus. Hematoxylin and eosin; × 110.

Fig. 11.—Fat in neurofibromatous tumor of vagus nerve. Region of left carotid sheath and nervus vagus. Hematoxylin and eosin; × 120.

node was seen in the submucosa of the ileum; it was surrounded by lymphoid and osteoid tissue and contained chitinous material. The scars in the right kidney were narrow and wedge-shaped, probably secondary to vascular obliteration. The pituitary gland was severely congested, and chromophobe cells predominated.

Summary of Pathological Findings

Neurofibromatosis with much neurilemmocytosis, hemangiomatosis, and glomangiomatosis, involving the left neck region, mediastinum, and upper abdomen; marked medial degeneration of the aorta and left carotid arteries; schwannomatosis and hemangiomatous involvement of the adventitia and vasa vasorum of the left neck arteries and thoracic aorta; hypoplasia of the aorta and large elastic arteries with mild aortic atherosclerosis and moderate atherosclerosis of the left carotid artery; status following attempted surgical control of

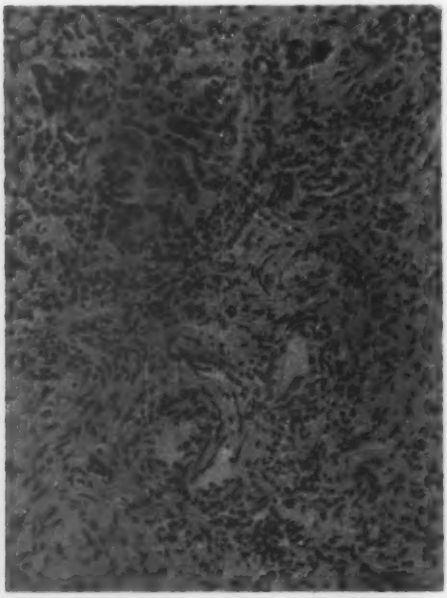


Fig. 12.—Thick-walled vessels in hamartomatous nodules in liver. Hematoxylin and eosin; × 120.

Fig. 13.—Neurofibromatous proliferation in the adventitia and focal thickening in the intima in the surgically removed portion of the left carotid artery. Hematoxylin and eosin; reduced to 92% of mag. × 65.

bleeding; hypoplasia of the liver, kidneys, and thyroid gland; bilateral pulmonary edema and congestion; acute congestion of the liver and spleen; hamartoma of the liver; vascular scars in the right kidney; single pigmented gallbladder stone; lobular hyperplasia of the breast, and chronic parasitic granuloma of the ileum.

Comment

The main histological features of interest in this case are the differentiation of organoid complexes as intrinsic components of the neurofibromatoid tissue, the presence of glomus-like structures and glomus-cell proliferation, and the medial sclerosis of the blood vessels in the involved areas.

Organoid Complexes

Whereas some pathologists [1,3,15,20] believe that neurofibromata originate exclusively from the endoneurial connective tissue sheath, others [4,9,11,21] derive these lesions from the neuroectodermal sheath of Schwann. Inglis [6] even used the term neurilemmoblastosis to describe neurofibromatous lesions characterized by the presence of cells regarded as of Schwannian lineage. In this connection it must be remembered

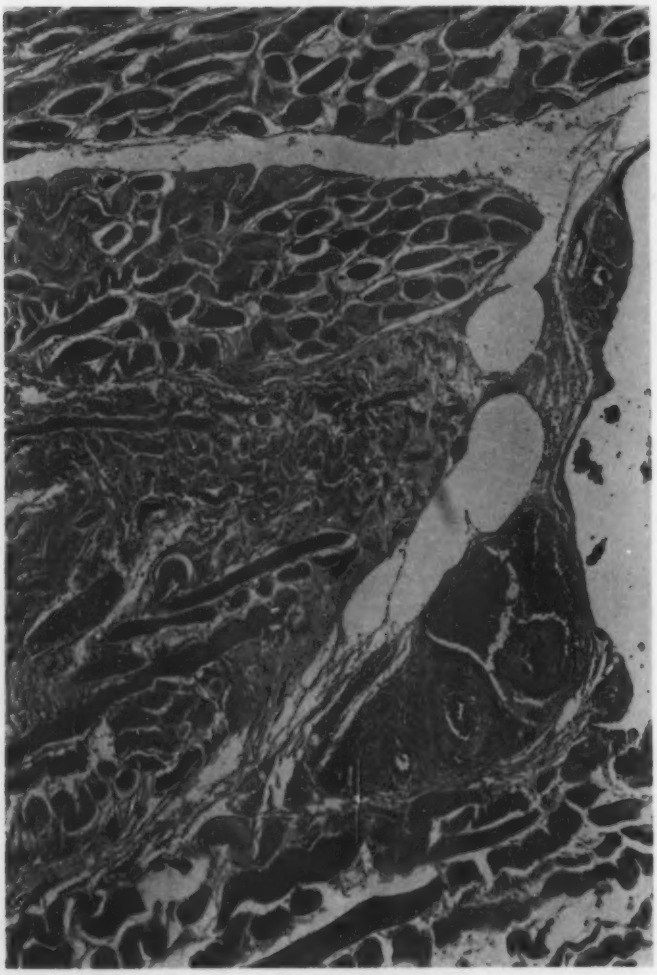


Fig. 14.—Neurofibromatous and angiomatous tissue in the left sternocleidomastoid muscle. Surgical excision in December, 1958. Hematoxylin and eosin; reduced to 92% of mag. × 65.

that Schwann cells not only are capable of forming fibroblastic tissue but possibly also determine and induce the development of the endoneurium; in fact, Murray and Stout,[14] as a result of tissue culture studies, concluded that the endoneurium arises partly from neuroectodermal cells. Whatever the precise histogenetic relationship of these two sheaths, endoneurium and neurilemma, there is no theoretical objection to their forming a common hamartomatous blastema in Recklinghausen's neurofibromatosis, and the intimate mixture of fibroblastic and neurilemmal differentiation in this case (Figs. 3, 4, and 8) seems to suggest such an event.

Cytologically, the Schwann cell and the fibroblast are not distinguishable by stains, and the extent to which cells of neuroectodermal origin participate in neurofibromatosis not only varies considerably but is difficult to estimate unless there is clear evidence of cellular regimentation and the differentiation of organoid bodies, viz., *lames foliacées*, Verocay bodies, or Meissneroid complexes. The palisaded elements are produced by neurilemmal proliferation, and the organoid structures are considered to represent the Schwannian supporting cells of

tactile corpuscles, particularly Wagner-Meissner corpuscles.[12,16]

Although Pacinian-corpuscle-like structures are present frequently in both ganglioneuromatous and neurofibromatous lesions in ducks,[19] readily recognizable caricatures of tactile organs seldom are encountered in neurofibromata in man. The Pacinian neurofibroma described by Prichard and Custer [18] probably represented only whorls produced by proliferating Schwann cells, as in Figure 4, but the rounded laminated structures elaborated in the neurofibroma reported by Cammermeyer [2] very likely indicate attempts at differentiating Pacinian-like corpuscles. Despite uncertainty in the interpretation of such organoid elements, their importance lies in that they constitute evidence of the participation of Schwann cells in neurofibromatosis.

Glomus-Cell Proliferation

There can be no doubt of the neurofibromatous nature, in this case, of the primary, essential, and dominant lesion with its varied histological appearances (Figs. 1-11). The intimately associated glomus-like arteriovenous shunts (Fig. 9), glomus-cell proliferation, and medial sclerosis of the vessels, however, constitute distinctly unusual manifestations of neurofibromatosis.[23] Although many vessels are merely dilated and congested, numerous others show considerable glomus-cell proliferation (Fig. 8), and the microscopic features suggest that not only the innervation of glomera but also the neural plexuses and terminal nerve twigs—which form a wide-meshed plexus in the outermost parts of the adventitia, a finer plexus in the junctional zone between the adventitia and the media, and a still more delicate one within the media [13]—of all the vessels, except capillaries, in the affected regions are involved by the neurofibromatous process.

The long indolent history of the original birthmark and the apparent extension of the lesions from this area suggest the existence of a hypothetical basic dysontogenetic anomaly affecting not only the nerve sheaths but vessels, glomera, and possibly bone in the involved areas. One of the generally accepted functions of glomera is to promote a flow of blood directly from an artery back into a vein. Glomus cells, which can be regarded as undifferentiated, nonfibrillated smooth muscle cells, however, are not necessarily concerned with diversion of blood; nevertheless, if they are given the requisite neural upset, might not their proliferation, as in the present case, be interpreted as a reactive one, attempting to control the vessel caliber?—an effort which ultimately failed when the hemorrhage became uncontrollable, itself an extraordinary feature of neurofibromatosis. Such a theory, essaying to explain the curious mixture of histological features, is conjectural, and it is realized that whoever enters the field of speculation in morphology knowingly ventures on very thin ice.

Summary

The histological features are described of a case of neurofibromatosis in which the patient died as a result of spontaneous, uncontrollable hemorrhage. Organoid complexes (Verocay bodies) occurred as intrinsic components of the neurofibromatoid tissue, which was itself intimately related to glomus-like structures, glomus-cell proliferation, and medial sclerosis of the vessels in the involved region.

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The Association of Idiopathic Hemochromatosis and Excessive Iron Overload

Report of a Case, with Comment Relative to the Concept of Exogenous Hemochromatosis

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Disorders related to iron storage have been the subject of much discussion since Sheldon's classical studies of hemochromatosis in 1935.[1] Much of this has been concerned with the validity of an exogenous form. However, approximately 80 examples of the latter resulting from either large numbers of blood transfusions or the excessive oral intake of iron preparations have been recorded up to 1956.[2] Many of these appear well documented. Some of the anatomical features utilized to distinguish primary or idiopathic hemochromatosis from that resulting from exogenous iron overload have been attributed to differences in portal of entry, quantity of iron involved, or duration of overload.[2] Only recently has a condition simulating exogenous hemochromatosis been produced in rabbits following the administration of excessively large quantities of colloidal iron.[3] Both forms of hemochromatosis are to be distinguished from the clinically insignificant deposition of iron pigment unassociated with morphologic alteration commonly referred to as hemosiderosis. The latter, which may represent a stage in the development of hemochromatosis, as indicated above, is common, whereas cases of exogenous hemochromatosis are comparatively rare. Because of this striking difference in the incidence of simple hemosiderosis and exogenous hemochromatosis, one might cogently inquire whether factors other than those noted might not be important in the pathogenesis of so-called exogenous hemochromatosis.

Recently, we have encountered a patient who exhibited many of the clinical features of hemochromatosis which appeared related to the ingestion of large quantities of iron in the form of ferrous sulfate over a 12-year period. Autopsy disclosed pathologic features of both primary and exogenous hemochromatosis. Investigation of the patient's sister and two daughters revealed hyperferremia, although they had not been exposed to exogenous supplies of iron save that present in a normal diet. In addition, one brother had succumbed after an illness which could have represented a primary hepatoma that had developed in the cirrhotic liver of hemochromatosis. These findings indicate that this case may represent the fortuitous association of idiopathic hemochromatosis of the familial type in a patient subjected to a marked iron overload. An alternative interpretation would suggest that the development of hemochromatosis was dependent upon iron overload in a patient susceptible to the development of this disorder.

Report of Case

A 67-year-old white man was admitted to the Veterans Administration Hospital, Pittsburgh, complaining of weight loss, weakness, dysuria, and diarrhea. He had lost 24 lb. in the preceding eight months. Weakness had been present for three years, and episodes of dysuria and diarrhea were first noted 12 years prior to admission. He was treated at that time for urethral stricture by transurethral resection and was placed on sulfonamide therapy, which the patient had been self-administering for the past 12 years. Similarly,

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he was taking an average of 10 capsules of ferrous sulfate (1,000 mg.) daily and varying amounts of cyanocobalamin U. S. P. (vitamin B_{12}) and ascorbic acid U. S. P. (vitamin C) for this length of time. He had been hospitalized one year previously for similar complaints. At that time hepatosplenomegaly and pancytopenia were observed. A clinical diagnosis of Banti's syndrome prompted surgical removal of the spleen. However, he showed little improvement following operation.

The patient's brother had been operated upon two years previously because of a mass in the right upper quadrant and jaundice, accompanied by a 50 lb. weight loss. Laparotomy revealed advanced cirrhosis of the liver and a hepatic mass considered to be a hepatoma. Unfortunately, no biopsy or autopsy was performed when he died, several months later. Serum iron determinations which were performed on the patient's sister and two daughters were 187μg., 176μg., and 140μg. per 100 ml., respectively. Routine blood studies failed to reveal other abnormality.

Physical examination revealed a well-developed but poorly nourished 67-year-old white man, who appeared chronically ill. His pulse was 110 per minute, and regular, and his blood pressure was 110/54 mm. Hg. His skin appeared slate-gray, and no angiomata were noted. There were no audible breath sounds over the left chest. The edge of the liver was palpable 5 fingerbreadths beneath the right costochondral margin, and was smooth, nontender, and firm. A feminine distribution of body hair was apparent, and the testes were atrophic.

Hemoglobin and red blood cell determinations, done on numerous occasions, varied from 9.5 to 10.5 gm. per 100 ml. and from 2,900,000 to 3,500,000 cells per cubic millimeter, respectively. His hematocrit was 35%, and leukocyte counts averaged 5,000 per cubic millimeter. Neutrophils varied from 30% to 41%; lymphocytes, 52% to 68%, with 5% monocytes and 2% eosinophils. Erythrocytes exhibited moderate basophilic stippling and anisocytosis. A siderocyte count of 9% was noted. Platelet counts averaged 128,000 per cubic millimeter, and reticulocyte counts, 1.9 per cubic millimeter. Coombs tests were negative, and corrected sedimentation rates, 26 mm. in one hour. A glucose tolerance test was normal, as were serum electrolytes. Total serum protein was 6.1 gm. per 100 ml., with 2.7 gm. albumin and 3.4 gm. globulin, per 100 ml. Urinalysis on occasion revealed a trace of reducing substance and numerous leukocytes. Thymol turbidity was

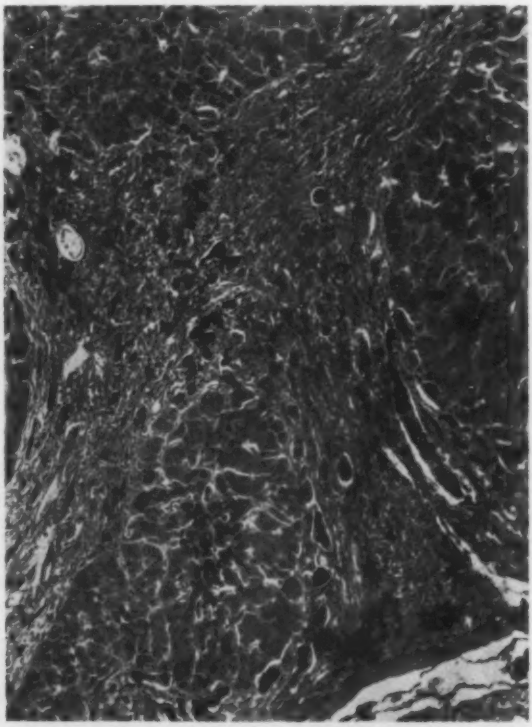

Fig. 1.—Distortion of hepatic lobular architecture with nodule formation. Abundant hemosiderin (appearing black) is evident in portal areas, Kupffer cells, and hepatic cells. Perls' reaction; × 140.

Fig. 2.—Hemosiderin (appearing black) is evident in epithelial cells of ducts within portal areas. Perls' reaction; × 180.

6.6 units; cephalin flocculation, 2+ in 48 hours. Serum bilirubin was never higher than 1.0 mg. per 100 ml., indirect. Sulfobromophthalein (Bromsulphalein; BSP) tests revealed 22% and 9% retention. Prothrombin time was 15 seconds (control, 13 seconds). Fecal urobilinogen was 180 Ehrlich units (E.U.) and urine urobilinogen 0.37 E.U. Bleeding and coagulation times were normal. Bile was absent from the urine. Urine cultures exhibited Escherichia coli and Aerobacter aerogenes, sensitive to chloramphenicol U. S. P. (Chloromycetin) in vitro. Watson-Schwartz tests of the urine for porphobilinogen were negative. Serum iron was 164μg. per 100 ml. The erythrocyte fragility test was within normal limits. Serologic tests for syphilis were negative.

Despite antibiotic and other supportive measures, the patient exhibited an ingravescent course and died several months after admission.

Pathologic Findings

Gross Examination

Macroscopic examination revealed extensive pleural adhesions on the left. The pulmonary parenchyma was firm and reddish purple. The liver weighed 3,000 gm. Its outer and cut surfaces were granular and reddish brown. The pancreas was small, firm, and rusty-brown. Many abdominal lymph nodes were similarly colored. The kidneys were large, each weighing 250 gm. The cortical surfaces were studded with small abscesses. Several calculi were present in the pelvis of both kidneys. The urinary bladder contained several calculi, and its wall was thickened and mucosa red and granular. The adrenal cortices were brown. The testes were small.

Microscopic Examination

Sections of lungs revealed that many alveoli contained purulent exudate. The pleural surface of the left lung was thickened, due to the presence of moderately cellular fibrous connective tissue containing focal collections of lymphocytes, plasma cells, and neutrophils.

Sections of the liver revealed distortion of hepatic lobular architecture, due to the presence of relatively acellular connective tissue in the portal and perilobular stroma. In many areas hepatic lobules were converted into nodules with markedly eccentric

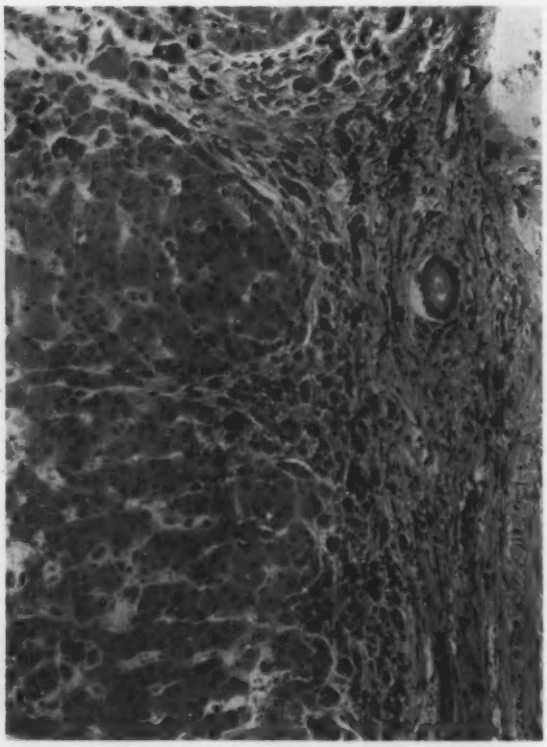

Fig. 3.—Hemofuscin (appearing black) is present in cells and stroma of portal areas, as well as in a few hepatic cells. Some light-gray granules of hemosiderin are evident also. Mallory's hemofuscin stain; × 180.

or imperceptible central veins (Fig. 1). This stroma contained collections of lymphocytes, and rarely neutrophils. In addition, ductular budding was evident, particularly in portal zones. The hepatic cells appeared uniform, although many contained brownish-yellow, granular pigment within their cytoplasms. This material exhibited a positive reaction for iron when sections were stained by Perls' method. This pigment was also found in abundance in the Kupffer cells and in the cytoplasm of ductular epithelium (Fig. 2). Hemofuscin pigment could also be demonstrated in the latter, as well as hepatic cells, fibrous stroma, and walls of blood vessels, by treating sections with basic fuchsin according to the method of Mallory [4] (Fig. 3).

Sections of pancreas revealed much interstitial fibrosis (Fig. 4). The cytoplasm of acinar and, to a less degree, of islet cells and macrophages within the pancreatic stroma contained hemosiderin. Hemofuscin was not identified. Squamous metaplasia of ductular epithelium was also present in some areas.

Hemosiderin-laden macrophages were numerous in lymph nodes.

Some cells of the zone glomerulosa of the adrenal cortices also contained hemosiderin, and this pigment was also found in synovial lining cells, as well as lamina propria of this structure.

The kidneys contained broad areas of neutrophilic and lymphocytic infiltrate with destruction of renal parenchyma. A similar inflammatory reaction was apparent within the mucosa and wall of the urinary bladder.

Sections of testes revealed marked anakmesis and hypoplasia of the gametogenic elements.

Hyperplasia of all cellular elements of the bone marrow was present in sections of this structure.

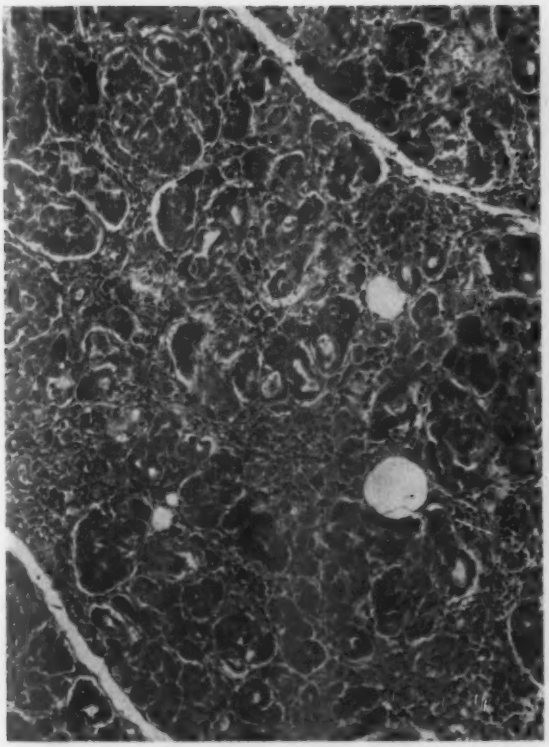

Fig. 4.—Fibrosis of pancreas is evident. Hemosiderin pigment (appearing black) is present in stroma and acinar cells. × 140.

Sections of the skin were not remarkable.

Examination of sections of spleen removed one year previously disclosed marked hemosiderin deposition.

Comment

The pathologic findings in this case correspond to descriptions of both primary and exogenous hemochromatosis. The metallaxis of liver and pancreas with abundant hemosiderin deposition in these organs represent salient features of the primary type. The presence of hemofuscin has been considered the *sine qua non* of primary hemochromatosis.[5,6] In addition, the presence of hemosiderin within lining cells of synovial membrane has been regarded as characteristic of primary hemochromatosis.[7] Although the diagnostic significance of hemosiderin in hepatic ductular epithelium has been questioned, our own experience has revealed this to represent a consistent finding in idiopathic hemochromatosis, as opposed to simple hemosiderosis or iron overload.[8] On the other hand, the marked deposition of hemosiderin in such reticuloendothelial sites as spleen and lymph nodes corresponds to the distribution of this pigment in exogenous hemochromatosis.

These findings in association with the familial evidence of hyperferremia suggest that this case is an example of idiopathic hemochromatosis of the familial type[9,10] in a patient who coincidentally received an excessive iron overload. In addition, it is of interest that he exhibited a refractory anemia of the pseudoaplastic type and severe renal infection, and also took large quantities of ascorbic acid, all factors considered to enhance the intestinal absorption of iron. It is also possible, however, that this patient, as well as his family, exhibited a susceptibility to the development of hemochromato-

sis, as evidenced by their hyperferremia, and that excessive iron overload was essential to the provocation of overt hemochromatosis in this patient. The absence of hemochromatosis in the patient's sister and daughters is in keeping with the rarity of this disorder in the female, although at least two are past the menopause. Further study of these family members should be highly informative. Unfortunately, sufficient details concerning the true nature of the brother's illness, which may have represented hemochromatosis, are lacking. Both interpretations, however, indicate that at least in some instances exogenous hemochromatosis may actually represent the very "remote possibility" of the coincidental association of idiopathic hemochromatosis and iron overload or that the development of exogenous hemochromatosis is dependent upon a susceptibility which might be disclosed in some instances by family study. Both concepts would offer an explanation for the relative rarity of exogenous hemochromatosis.

Summary

A case of hemochromatosis in a 67-year-old white man who received an excessive iron overload over a 12-year period, as well as demonstrating other features recognized to enhance iron absorption, is presented. Pathologic study disclosed evidence of both idiopathic and exogenous hemochromatosis. The demonstration of hyperferremia in a sibling and two daughters strongly suggests that this case represents the occurrence of idiopathic hemochromatosis of the familial type in a patient coincidentally subjected to iron overload. An alternative interpretation indicates that the latter was essential for the provocation of overt hemochromatosis in a patient exhibiting an inherent susceptibility to this disorder.

These interpretations appear significant concerning the frequency, as well as the concept, of exogenous hemochromatosis.

The authors wish to thank Dr. Wallace Jensen for the determinations of serum iron.

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Hypercholesteremia in the Prairie Gopher (Ground Squirrel)

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Since Ignatowski and Anitschkow found that feeding of cholesterol produces in rabbits vascular lesions which resemble atherosclerotic changes in man, many other animal genera have been examined as to their susceptibility to experimental cholesterol atherosclerosis. Guinea pigs responded quite readily, whereas monkeys, dogs, cats, rats, and mice did not. Later on it was shown that dogs became susceptible if made hypothyroid; alterations of coronary arteries and aortas in rats could be produced by upsetting dietary balances, and monkeys could be made atherosclerotic by a diet deficient in pyridoxine. Recently swine were made atherosclerotic when fed butter.[9] Altschul[2] has shown that golden (Syrian) hamsters can be made atherosclerotic by feeding them egg yolk and milk. In these animals it was found that, in addition to lesions of the vessel wall proper, thrombosis also occurred, which had never been reported in experimental atherosclerosis, for which reason some authors had hesitated to consider experimental atherosclerosis as equivalent to the human type. Birds (chickens, pigeons, parrots, and parakeets) were rendered atherosclerotic by high intake of cholesterol, but there are apparently strong differences as to susceptibility, depending on the strain used. Geese and ducks were made atherosclerotic by forced feeding of a diet rich in carbohydrates.[11] For more details on species- and genus-bound differences in susceptibility to experimental cholesterol atherosclerosis, one may consult the reviews by Duff,[5] Cook,[4] and Kritchevsky.[7]

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In 1950 Altschul[1] reported that the prairie gopher (correct name: ground squirrel; Citellus richardsonii, Sabine) did not develop experimental atherosclerosis if fed exclusively egg yolk and milk for up to seven months. At that time, no serum cholesterol was determined. Also, in 1950, Wilber and Musacchia[10] reported that the cholesterol of whole blood in 10 normal Alaskan ground squirrels (Citellus barrowensis) was 224 mg. %, without apparent seasonal variations. Later, Bragdon[3] investigated Citellus columbianus, which belongs to the same genus as Citellus richardsonii, but to a different species. In these animals the average serum cholesterol was 215 mg. % after one to three months in captivity on a low-fat vegetable diet. When the animals were kept on a high-fat vegetable diet for two to eight months, the serum cholesterol rose to 324 mg. %, whereas four other animals, when fed rabbit pellets containing 1% cholesterol for four weeks, showed a serum cholesterol of 277 mg. %. The morphological changes in Bragdon's animals (intracellular fat deposits in those animals which had hyperlipemia) were apparently not related to cholesterol intake.

In view of the fact that in Altschul's previous experiments no cholesterol levels had been determined, that in Wilber and Musacchia's investigation no cholesterol had been fed, and that Bragdon had fed only a moderate quantity of cholesterol to five of his animals for only four weeks, we decided to determine the serum cholesterol in normal prairie gophers (Citellus richardsonii) and also in animals given cholesterol in their feed for various periods. In addition, morphological changes in blood

TABLE 1.—*Serum Cholesterol in Normal Prairie Gophers (Ground Squirrels) After Several Weeks in Captivity on Calf Meal Pellets and Oats ad lib.*

Animal No.	Serum Cholesterol, Mg. %	
1	231	In fall
2	255	
3	214	
4	523	
5	238	In spring
6	155	
7	258	
8	221	
9	240	
10	288	
Average	262.6 mg. %	

vessels and various organs were to be looked for.

Material and Methods

In 10 healthy prairie gophers (Citellus richardsonii, Sabine) serum cholesterol was determined (method of Schönheimer-Sperry, as modified by C. S. McArthur), on four animals in fall and on six in spring. The animals had been captured in the vicinity of this university and kept for several weeks on a diet of calf-meal pellets and oats before blood samples were taken.

Of another group, of 22 gophers, 2 were fed ad libitum calf meal pellets containing 3.6% cholesterol, and 20 animals received these pellets, also ad lib., but in addition an egg yolk cake (Altschul,[1] 1950) and milk. The animals liked both these diets very much and grew very fat, in contrast to rabbits, which after comparable times on such diets, become very lean, and even emaciated. None of the cholesterol-fed gophers died spontaneously. They were killed after periods ranging from 63 to 297 days. Prior to death, blood was taken from the heart, and the serum cholesterol was determined. In two cases the cholesterol in beta-lipoproteins was also determined (Dr. L. Horlick). The animals were dissected, examined macroscopically and tissues (brain, eyes, tongue, thyroid, aorta, heart, spleen, liver, parts of the gastrointestinal tract, kidneys, adrenals, and gonads) were prepared for microscopical studies.

TABLE 2.—*Serum Cholesterol and Tissue Changes in Prairie Gophers Treated with Cholesterol*

Animal No.	Days	Serum Cholesterol at Time of Death, Mg. %	Vascular Lesions	Changes in Other Tissues
		Calf Meal Pellets with 3.6% Cholesterol ad lib.		
1	117	807	--	Glomerulonephritis
2	117	2,724	--	Cirrhotic liver
		Calf Meal Pellets with 3.6% Cholesterol ad lib. and Yolk Cake and Milk ad lib.		
3	63	1,151	--	Liver fat increased; a few foamy epithelial cells in renal cortex
4	63	1,620	--	Liver fat increased
5	64	3,172	--	" " " ; some lipidosis of suprarenal cortex
6	64	1,309	--	" " "
7	64	516	--	" " "
8	91	1,350	--	Liver fat slightly increased
9	91	1,350	--	--
10	101	394	--	--
11	101	1,093	--	--
12	101	793	--	--
13	109	573	--	Liver fat slightly increased
14	109	708	--	--
15	146	1,360	--	Liver fat increased; slight liver cirrhosis
16	146	1,104	--	A few fatty cells in adrenal cortex; glomerulonephritis
17	146	2,058	--	Liver fat slightly increased
18	256	1,145	+ *	" " " " ; slight liver cirrhosis
19	256	962	+ †	Liver fat increased; liver cirrhosis
20	256	785	--	Liver fat increased; slight liver cirrhosis; cholesterol crystals in pericardium
21	293	981	--	Liver fat increased; nephritis
22	297	4,078	--	" " "

* One small atheromatous focus in the aorta.
† One small atheromatous focus in the aorta; one small branch of coronary art. atheromatous

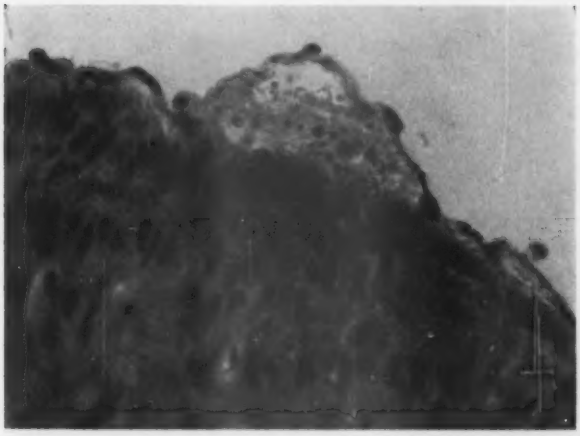

Fig. 1 (Case 18).—Aorta of prairie gopher with minute atheromatous lesion. Iron hematoxylin-eosin stain; magnification × 500.

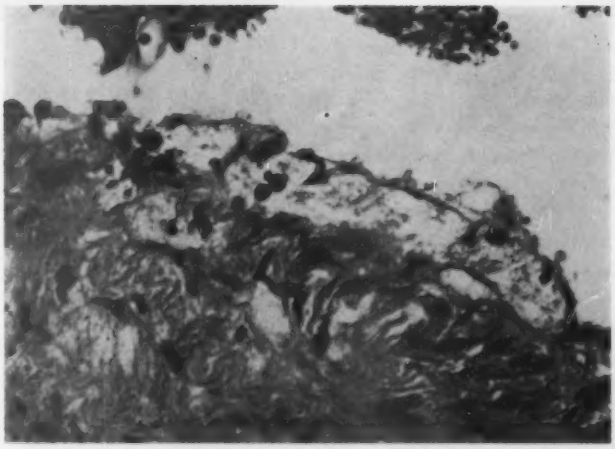

Fig. 2 (Case 19).—Aorta of prairie gopher with foam cells in and below endothelial lining. Hematoxylin-eosin stain; magnification × 500.

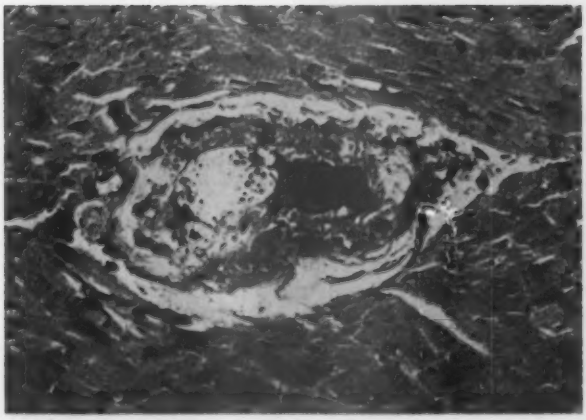

Fig. 3 (Case 19).—Myocardium of same animal as that in Figure 2, with atheromatous branch of coronary artery. Hematoxylin-eosin stain; magnification × 266.

Results

The serum cholesterol levels of 10 normal ground squirrels (Citellus richardsonii, Sabine) are given in Table 1; those of 22 other animals, fed cholesterol for various periods, in Table 2, in which table the tissue changes are also registered. Table 2 shows that, although in a number of cases the liver showed excess of fat and various stages of cirrhosis, and some other organs showed minor changes, there were only two animals with vascular lesions, both animals having single foci in the aorta and one of them having a lesion in a small branch of the coronary artery (Figs. 1, 2, and 3).

The cholesterol in the beta lipoprotein fraction of the serum in the animal (No. 22) with a serum cholesterol of 4,087 mg. % was 73.5%; in the animal with a serum cholesterol of 981 mg. % it was 80.3%, which indicates that there was no shift toward the alpha lipoproteins.

Comment

The following findings appear noteworthy:

1. The normal animals have a relatively high serum cholesterol. It exceeds all averages quoted for many other genera in the reviews by Cook [4] and by Kritchevsky.[7] It is also higher than the average serum cholesterol in healthy humans. Wilber and Musacchia's,[10] and Bragdon's [3] findings on different species of the same genus are also high if compared with other animal genera.

2. There is a relatively early rise of the cholesterol level in the experimental animals.

3. Even after prolonged treatment the serum cholesterol levels remain high, whereas in cholesterol-fed rabbits, for instance, the serum cholesterol rises in the first two months but decreases in the third and later months (Hueper [6] and other authors).

4. Only 2 out of 22 experimental animals showed vascular lesions, which were, moreover, discrete. They occurred neither in the animals with the highest cholesterol levels nor in the animals which were kept longest on the cholesterol diet.

5. In spite of the high serum cholesterol, the body fat was not depleted, as occurs in cholesterol-treated rabbits, in which, according to Popjak,[8] the body fat becomes mobilized to keep the plasma cholesterol in suspension.

It is not surprising that there were neither correlations between the duration of the treatment and the intensity of cholesteremia nor similarity between the single animals in their reaction to the cholesterol feeding. This "unruliness" is also found in rabbits.

Comment

It seems to us that the fundamental importance of our finding lies not so much in the high serum cholesterol of normal prairie gophers or in the ease with which it can be raised by feeding them cholesterol, but in the fact that in spite of intense hypercholesteremia vascular changes were missing in 20 animals and were only slight in 2. It may, therefore, be concluded that in Citellus richardsonii the vulnerability of the vascular system to hypercholesteremia is very slight or missing, thus supporting the view that in other genera and man, hypercholesteremia is the secondary, not the primary, factor in atherogenesis.

Summary

The serum cholesterol of the normal prairie gopher (ground squirrel; Citellus richardsonii, Sabine) is high if compared with that of other genera. Although the serum cholesterol could be raised very much by feeding cholesterol to the animals, only small vascular lesions were found in only 2 out of 22 animals, in spite of the fact that some of the animals were kept hypercholesteremic for up to 297 days and the serum cholesterol had risen up to 4,078 mg. %.

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The Comparative Pathology of Goiter in a Nonendemic and an Endemic Area

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During the past two years we have collected thyroids at consecutive autopsies from each of our respective areas, New Orleans, and Cali, Colombia. When these collections were compared with each other, it became apparent that there were differences in distribution of morphologic types of nodular goiter from these two regions. The goiters from Cali seemed to have a far greater incidence of compact cellular "parenchymatous" nodules than did New Orleans goiters, in which the colloid nodule was predominant. Since New Orleans is considered to be in a nonendemic area [1,2] and since Cali is in a low-iodine, endemic goiter area,[3] it is felt that these morphologic differences should be described, since they emphasize that nodular goiter may not always represent the identical pathologic process as seen from one region to another. This would have bearing on etiologic factors, and would be important in the consideration of potentialities for malignant change. At the present time there is very little in the literature concerning the differences in the pathology of nodular goiter as seen in endemic and nonendemic areas, and comparisons can be made between reported collections only on the basis of overall incidence, not on pathologic differences.

This comparison involves primarily a study of colloid goiter, both the diffuse form and the nodular form, in the young adult and in older age groups. We do not include the diffuse hyperplastic goiter of hyperthyroidism, since this is not a problem considered related to geographical factors.

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Materials and Methods

Consecutive thyroid glands were collected from autopsies at Charity Hospital of New Orleans; the Coroner's Office, Orleans Parish; the Hospital Universitario, and the medicolegal office at Cali, Colombia. The two hospitals are of similar type, drawing a wide variety of acute and chronic diseases from persons in a lower economic status. There were a total of 250 thyroids from persons over 15 years of age from New Orleans, as compared with 163 thyroids of similar selection from Cali. From these, there were 63 goiters from New Orleans and 62 from Cali.

All thyroids were weighed, fixed in 10% formalin, and serially sectioned at about 2 mm. thickness. Blocks from representative areas were taken for histologic study, and the sectioned glands were laid out in plastic bags and sealed according to the method of Holman et al. (Fig. 1).[4] The glands were then classified into one of six categories, on the basis of the type of nodules present, whether a solid parenchymatous type or a translucent colloid type. Many of the glands, while markedly nodular, were not enlarged and weighed less than 35 gm. These smaller glands may not have presented clinically as an enlargement or goiter; yet they represent the same pathologic process as that in the larger glands. On this basis we had seven categories: (1) diffuse colloid goiter; (2) colloid nodular goiter; (3) parenchymatous nodular goiter; (4) mixed colloid-parenchymatous nodular goiter, colloid predominant; (5) mixed colloid-parenchymatous nodular goiter, parenchymatous nodular type predominant; (6) mixed colloid-parenchymatous nodular goiter, none predominant; (7) unclassified.

1. The diffuse colloid goiter was defined as an enlarged gland weighing over 35 gm., having no colloid nodules greater than 1 cm. (Fig. 2).

2. The colloid nodular goiter contained varying numbers of purely colloid nodules, one of which was required to be over 1 cm. in diameter (Fig. 3). Colloid nodules were grossly translucent and microscopically contained mostly large follicles

filled with colloid. Central hyalinization and calcification was not an uncommon feature of this type of nodule.

3. The parenchymatous nodular goiter contained varying numbers of purely parenchymatous nodules, only one of which was required to be over 1 cm. in diameter (Fig. 4). Parenchymatous nodules

Fig. 1.—Thyroid gland sectioned and mounted in plastic bag.

were recognized grossly as opaque, grayish-white areas, which microscopically proved to be of a variety of types. These different types were a compact fetal type, embryonal type, Hürthle type, and mixtures of the same. The nodules were

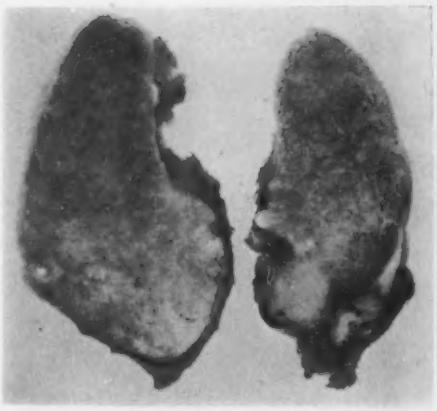

Fig. 2.—Diffuse colloid goiter, transilluminated. Many of the transparent, enlarged follicles appear as white spots (New Orleans A58-185).

usually well demarcated from the surrounding parenchyma, and a fibrous capsule was variably present.

4, 5, 6. The mixed colloid-parenchymatous nodular goiter was a gland in which both types of nodules were present, or individual nodules contained a mixture of the two types (Fig. 5). This was subdivided into three groups, depending on the predominance of the type of nodule. Thus, there were the colloid-predominant, the parenchymatous-predominant, and none-predominant groups.

7. In the unclassified group were enlarged glands, over 35 gm., in which there was no evidence of nodularity or excessive follicle involution. The glands on microscopic section would be considered as normal, but in view of their large size were included in the series as goiters.

Fig. 3.—Colloid nodular goiter, transilluminated to illustrate translucency of colloid nodules. Black spots are hemorrhagic areas. (New Orleans A58-605).

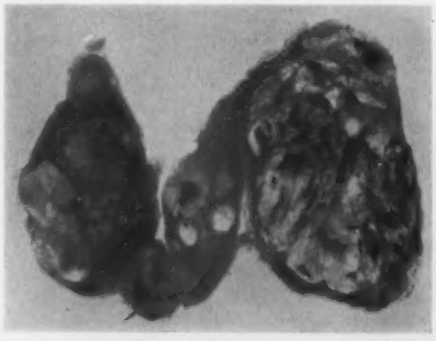

Fig. 4.—Parenchymatous nodular goiter, transilluminated to illustrate density of cellular, compact nodules (Cali ML-119).

Fig. 5.—Mixed colloid-parenchymatous nodular goiter, parenchymatous predominant, transilluminated. There is only one translucent colloid nodule (Cali ML-218).

Results

The number of goiters by age groups is given in Table 1. This may be graphed on the basis of per cent incidence within each age group (Fig. 6), giving a rough picture of the differences between the two areas. Goiter begins about a decade earlier in the Cali series and reaches a peak incidence of 57% in the 55-64 age group, dropping to 33% a decade later. There is only one patient in the 74-year-and-over group from Cali; therefore the results are incomplete at that point. The New Orleans goiters gradually increase to a peak of 45% in the 74-85 age group, dropping to 33% in the 85-year-and-over group. Thus, Cali is both earlier in onset of goiter and greater in incidence than New Orleans. Both areas show an increasing goiter incidence in the higher age group, but in the older extremes there is a diminution of incidence; this drop possibly is of no significance, owing to the numbers involved.

TABLE 1.—*Distribution of Goiters from New Orleans and Cali by Age Groups*

	Age Groups, Yr.	Total Thyroids	Total Goiters	Diffuse Colloid Goiter	Colloid Nodular Goiter	Parenchymatous Nodular Goiter	Mixed Types Goiter	Unclassified
New Orleans	15-25	10	0					
	25-34	12	1		1			
	35-44	21	5	3	2			
	45-54	45	8	1	4		3	
	55-64	52	13	4	5		4	
	65-74	67	16	1	9	1	6	1
	75-84	34	15		10		5	
	85-over	9	3	1	2			
Cali	15-25	55	5	1	2		2	
	25-34	49	12	4	1	4	2	1
	35-44	37	13	1	3	3	6	
	45-54	31	15	2	2	5	5	1
	55-64	14	8			3	4	1
	65-74	21	7			2	5	
	75-84	1	0					
	85-over	0						

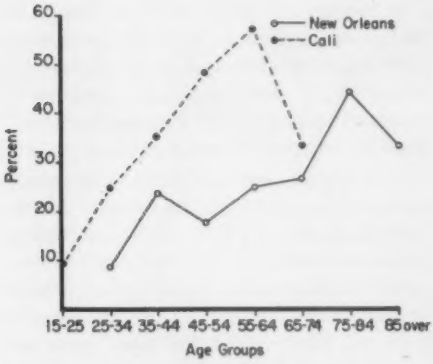

Fig. 6.—Per cent incidence of all types of goiter in New Orleans and Cali, by age groups.

The greatest difference in the distribution of types of goiter between the two groups is seen in the pure parenchymatous nodular goiter, in which Cali shows a marked preponderance (Table 2). The sum of all of the glands that show any degree of parenchymatous nodularity comprises 67.8% of the Cali series and 31.8% of the New Orleans series. Colloid nodular goiter occurs with much greater frequency in the New Orleans series. Diffuse colloid goiter and the colloid-predominant form of mixed nodular goiter occur with about equal frequencies in the two series.

From the data in Table 1 the per cent incidence by age groups of the various types of goiter can be graphed for a rough comparative picture, although the numbers are small and only broad interpretations can be made (Figs. 7, 8). Diffuse colloid goiter and nodular colloid goiter are not found in the Cali series after the age of 55, and the rise in over-all incidence of goiter after the age of 55 in Cali is therefore due to the combined increasing incidence of pure parenchymatous nodular goiter and the mixed goiters. In the New Orleans series, however, colloid nodular goiter is seen at a rising frequency on into the seventh and eighth decades, contributing significantly to the over-all incidence in the 74-85 age group. The mixed goiters in New Orleans gradually increase in incidence as the age groups progress, and comprise the remainder of goiters in the 74-85 age group. Within the group of mixed goiters none occur in the New Orleans series below the age of 35, whereas in Cali, mixed goiters begin in the 15-24 age group.

The over-all average weights of the goiters were 42.5 gm. for New Orleans, as against 53.6 gm. for Cali. The Cali goiters in almost every subgroup are significantly heavier (Table 3). Both New Orleans and Cali show a higher incidence of goiter in the female (Table 4). There

TABLE 2.—*Per Cent Distribution of Types of Goiter in New Orleans and Cali*

	Diffuse Colloid Goiter, %	Colloid Nodular Goiter, %	Parenchymatous Nodular Goiter, %	Mixed Colloid-Parenchymatous Nodular Goiter: Colloid Predom., %	Mixed Colloid-Parenchymatous Nodular Goiter: Parenchymatous Predom., %	Mixed Colloid-Parenchymatous Nodular Goiter: None Predom., %	Unclassified, %
New Orleans	(10)* 15.9	(33) 52.3	(1) 1.6	(15) 23.8	(0)	(3) 4.8	(1) 1.6
Cali	(9) 14.5	(8) 12.9	(17) 27.4	(15) 24.2	(4) 6.5	(6) 9.7	(3) 4.8

* Parentheses refer to number of glands in each group.

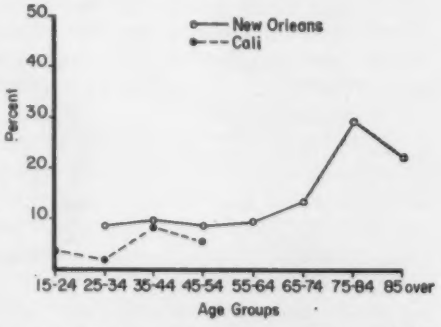

Fig. 7.—Per cent incidence of colloid nodular goiter in New Orleans and Cali by age groups.

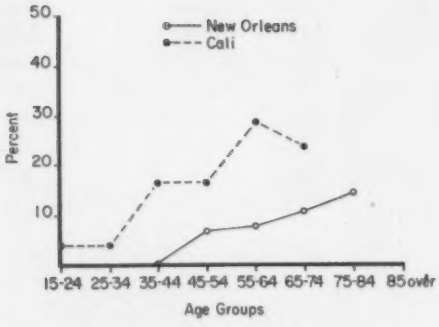

Fig. 8.—Per cent incidence of all mixed colloid-parenchymatous nodular goiters in New Orleans and Cali by age groups.

are racial differences between the two samples. The Cali series contains a number of mestizos, a racial mixture of white and Indian, not represented in the New Orleans group (Table 5). The New Orleans series contains more Negroes than Cali.

The method of storage in plastic bags after sectioning facilitates the comparative study. The bags can be readily handled during discussion of both the gross and the microscopic examination, and they can be manipulated quickly during classification procedures.

New Orleans is not considered an endemic goiter area, although some question has existed on this point. In 1929, Olesen [5] reported an incidence of goiter in parishes of southern Louisiana ranging from 35% to 50% of boys and girls in the age range of 6 to 18 years. This has been questioned subsequently by clinicians interested in thyroid disease in this area, on the basis that these figures were obtained by correspondence with state and parish health officers, and no statement was given as to the method of estimation.[6] The incidence of clinically evident goiter in New Orleans generally is agreed to be quite low as compared with

TABLE 3.—*Average Weights in Grams of Types of Goiter in New Orleans and Cali*

	Diffuse Colloid Goiter	Colloid Nodular Goiter	Parenchymatous Nodular Goiter	Mixed Nodular Goiter			
				Colloid Predom.	Parenchymatous	None Predom.	Unclassified
New Orleans	(10)* 37.0	(33) 44.1	(1) 10	(15) 50.0	(0)	(3) 26.6	(1) 55.0
Cali	(8) 62.1	(17) 54.1	(15) 42.4	(15) 70.6	(4) 62.5	(6) 26.0	(3) 58.6

* Parentheses refer to number of glands in each group for averages.

Comment

In our classification of these goiters, the subdivisions are strictly for the purpose of illustrating the morphologic variations, and we do not propose this as a solution to the classification of goiter. Using these subdivisions, however, there is good correlation after independently classifying each series.

TABLE 4.—*Sex Distribution of Goiters in New Orleans and Cali*

	Male			Female		
	Total Thyroids	Goiters	% Incidence	Total Thyroids	Goiters	% Incidence
New Orleans	151	32	21.2	99	31	31.3
Cali	115	38	33.0	48	23	47.9

that seen in unquestioned endemic areas. As can be seen from the present report, anatomic evidence of goiter is frequent, particularly in the older age groups. This discrepancy can be partly explained by the fact that 32, or over half, of the goiters in the New Orleans series weighed less than 35 gm. and probably were not clinically apparent. In addition, some of the glands over 35 gm. in weight may not have been palpable as a result of other factors, such as obesity or

TABLE 5.—*Racial Distribution of Goiters in New Orleans and Cali*

	White	Negro	Mestizo	Indian
New Orleans	19	44		
Cali	23	11	24	1

thick neck. Cali, however, is recognized as an endemic goiter area; the clinical incidence in school children is about 55%.[3] There are no clinical reports of the incidence of goiter in older adults. Only 16, or about one-fourth, of the goiters from the Cali series weighed less than 35 gm. In Cali there is known iodine deficiency in the salt, which is considered the cause of endemic goiter in the area.[3] New Orleans, a so-called nonendemic goiter area, is actually endemic by pathologic study in the older age groups; yet there is no significant iodine deficiency in the region to our knowledge.

The striking morphologic difference was the prominent parenchymatous nodularity in the Cali series. As diffuse colloid goiter and colloid nodular goiter disappeared after the age of 55 in Cali, parenchymatous nodular goiter took prominence. One might assume, then, that the nodular colloid goiter was merely progressing to mixed parenchymatous nodularity as aging progressed. However, this does not explain the process of colloid nodular goiter in New Orleans in which pure colloid nodularity persists and increases in incidence in later years. Although parenchymatous nodularity increases in New Orleans with age, it does not do so to such a degree as in Cali. Racial differences probably are not significant; of the group of 24 mestizos, 17 had some form of parenchymatous nodularity, no more than would be expected of their share in the distribution. Sex is not a factor in parenchymatous nodular change, since in the Cali series pure parenchymatous nodular goiter did not occur with any predominance in either sex; 10 glands occurred in men, and 7 glands occurred in women. This morphologic difference in the goiters is consistent with other known experimental data. In the experimental animal pure colloid nodular goiter has not been produced with simple iodine deficiency, but parenchymatous nodular goiter can be produced.[7] Also, in endemic goiter areas the introduction of iodine to the diet reduces the clinical incidence of goiter substantially, but not completely. In a so-called nonendemic area, such as New Orleans, the over-all incidence of goiter in adults in the autopsy series has run consistently above 20%. It may be that the colloid nodular goiter in New Orleans is initiated by a completely different etiology unrelated to deficiency of any type. Other dietary or constitutional factors can play a role in the production of this type of lesion. Iodine deficiency when present would most certainly aggravate such a process, yet not be the primary cause.

A comparison such as this brings out the difficulties encountered in understanding the goiter problem between pathologists from different regions. The "parenchymatous nodules" that we studied would be considered true adenomas occurring in nodular goiter by some, or as localized hyperplasias as a part of the general picture of nodular goiter by others. We do not feel that these parenchymatous nodules can be accurately termed adenoma or hyperplasia by our present morphologic methods and knowledge, and thus have used a noncommittal term. There is no question that the parenchymatous nodules are a part of the general picture of nodular goiter, but their relation to malignancy is not clear. Thus, in the New Orleans area a surgical specimen of a nodular, colloid goiter in which a single solid parenchymatous nodule is found would cause added concern and study regarding the presence of malignancy, especially if there were questionable capsular invasion. If the same gland were taken to an endemic goiter area and shown to a pathologist familiar with the disease in his area, he might tend to minimize any potential danger from the presence of such a parenchymatous nodule on the basis of his experience in which this feature is quite common; yet his incidence of deaths from carcinoma of the thyroid is no higher than that from nonendemic areas. Although, admittedly, there are rare instances in which this type of nodule metastasizes, the vast majority do not, and the truly malignant ones can only occasionally be diagnosed with certainty in the

absence of metastases. The cellular histology is usually benign, and moderate nuclear atypicalities with variation in nuclear size do not connote malignancy in this type of nodule. It is our belief that limited capsular invasion, without obvious distant invasion of the gland, is a common feature of the parenchymatous nodule of nodular goiter, and does not indicate malignancy. Many of the parenchymatous nodules are unencapsulated, and, of those that are surrounded by a capsule, the capsule may be incompletely developed in areas, leading to the impression of aggressive invasion. Only definite vascular invasion or lymph node metastases are reliable indicators of malignancy in this situation. To use less stringent requirements would result in an abnormally high incidence of carcinoma arising in nodular goiter, which is not consistent with the death rate from cancer of the thyroid in some endemic goiter areas.[8] In our group of 125 goiters, there were no cases of unequivocal carcinoma.

Summary

A comparative study of colloid goiter in the young adult and older age groups was made from thyroid glands obtained at autopsy from New Orleans, and Cali, Colombia, over a two-year period. The onset of goiter in Cali was at an earlier age, and the over-all incidence of goiter was much higher for each successive age group, as compared with that in New Orleans. There was a marked preponderance of compact, cellular "parenchymatous" nodularity in the Cali series. Pure colloid nodularity was not present after the age of 55 in Cali, when mixtures of colloid and parenchymatous nodularity or pure parenchymatous nodularity was found. In New Orleans, however, pure colloid nodularity continued at a rising frequency on into the seventh and eighth decades of life.

The relationship of iodine deficiency to the cause of these differences is discussed, and it is suggested that iodine lack may not be the primary cause of colloid nodular goiter in the New Orleans area. Iodine deficiency in Cali may be the cause of the increased incidence of parenchymatous nodularity.

The problems involved in the diagnosis of malignancy in parenchymatous nodules are discussed in relation to local experience with goiter pathology.

Department of Pathology, Louisiana State University School of Medicine (12).

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Carcinoid of Meckel's Diverticulum

Report of a Case and Review of the Literature

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During the past decade there has been great clinical, pathological, and biochemical interest in carcinoid tumors of the intestinal tract. These tumors arise from the argentaffin or chromaffin cells (also known as Kulchitsky cells) in the bases of the crypts of Lieberkühn throughout the gastrointestinal tract.[1,2] From 50% [1] to 90% [3] of these growths appear in the appendix, other sites being the ileum, colon and rectum, jejunum, duodenum, and stomach, in order of decreasing frequency.[1,4] Meckel's diverticulum is an infrequent site for this neoplasm. We wish to describe a case of carcinoid arising in a Meckel's diverticulum and give a short review of the 16 previously reported cases (Table).

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Report of Case

A 71-year-old white man had signs suggestive of a peptic ulcer for three years, but radiological studies of the upper gastrointestinal tract were normal. On April 22, 1959, he was hospitalized with an acute myocardial infarction. Anticoagulant therapy (heparin) was discontinued on April 23, when he vomited coffee-grounds material that was strongly benzidine positive. Over the next three weeks he became weaker, developed basilar rales in both lungs, showed a low-grade fever, and had electrocardiographic evidence of extension of the myocardial infarction. On May 11, thrombosis of the right popliteal artery occurred, and anticoagulant therapy (heparin) was reinstituted. The heparin was again stopped on May 14, when he vomited benzidine-positive coffee-grounds material. He remained weak; his blood pressure fell slowly,

Reported Cases of Carcinoid Occurring in Meckel's Diverticulum

Case No.	Author	Age, Yr.	Sex	Surgical or Necropsy	Clinical Symptoms	Metastasis	Tumor Size	Stain for Argentaffin Granules
1	Hicks & Kadinsky [6]	12	M	Surgical	Yes	None	Not stated	Not done
2	Stewart & Taylor [7]	54	M	Necropsy	No	None	Pea	Positive
3	Hertzog & Carlson [8]	54	M	Necropsy	No	None	4 mm.	Positive
4	Hertzog & Carlson [8]	58	M	Necropsy	No	None	3 mm.	Positive
5	Price [9]	54	F	Surgical	Yes	None	Small orange	Negative
6	Collins, Collins & Andrews [10]	56	M	Surgical	Yes	None	Small	Not done
7	Ashworth & Wallace [11]	46	M	Necropsy	No	None	8 mm.	Negative
8	Paulter & Scotti [12]	68	M	Necropsy	No	None	6 mm.	Positive
9	Mrazek, Godwin, & Mohardt [13]	37	M	Surgical	No	None	Not stated	Not done
10	Mrazek, Godwin, & Mohardt [13]	54	M	Necropsy	No	None	3 mm.	Not done
11	Pierce [14]	47	M	Surgical	Yes	None	Not stated	Positive
12	Grimes & Crane [15]	59	F	Surgical	Yes	Massive to liver	15 mm.	Not done
13	Stoll [16]	58	M	Necropsy	No	Solitary 5 mm. liver nodule	5 mm.	Positive
14	Parnassa, Friedman, & Cincotti [17]	56	M	Surgical	Yes	None	Not stated	Not done
15	Baird, Anderson, & Mills [18]	42	M	Surgical	No	None	4 mm.	Positive
16	Rintala [19]	25	M	Surgical	Yes	None	3 mm.	Negative
17	Drickman and Hodges	71	M	Necropsy	Yes	Mesenteric lymph nodes	8 mm.	Positive

Fig. 1.—Portion of ileum with Meckel's diverticulum, containing ulceration at arrow to right, and carcinoid at arrow to left.

and he died on May 18, or 26 days after his hospitalization.

Pathology

No evidence of mucosal ulceration was present in the esophagus, stomach, or duodenum. Autopsy revealed sclerotic occlusion of the anterior descending branch of the left coronary artery and a massive organizing anteroseptal myocardial infarction with mural thrombi adherent to the endocardium of the left ventricle. There were multiple renal and splenic infarctions and bilateral adrenal infarctions. Multiple small pulmonary infarctions and osteitis deformans of the skull were also noted. All of these findings were substantiated on histological examination.

Coffee-grounds material, mixed with loose feces, was present in the ileum. In the distal ileum, about 30 cm. from the ileocecal valve, a Meckel's diverticulum was located (Fig. 1). It measured 2 cm. in

Fig. 2.—Simple mucosal ulceration in Meckel's diverticulum; mag. × 50.

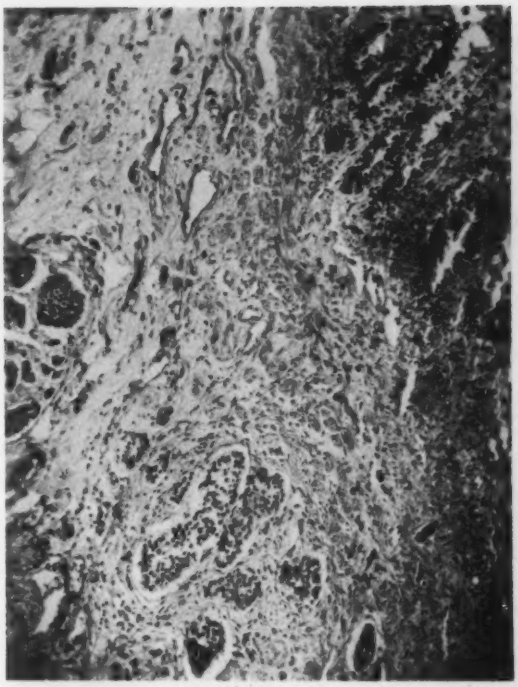

Fig. 3.—Carcinoid of Meckel's diverticulum; mag. × 100.

diameter and 4 cm. in length and was filled with loose feces and blood. Near the tip of the diverticulum two abnormalities were noted. One consisted of a mucosal ulceration, the edges of which were smooth, elevated, indurated, and edematous. Histologically, the defect extended to the level of the muscularis (Fig. 2). Its edges and the base were permeated with inflammatory cells but were devoid of tumor. The other

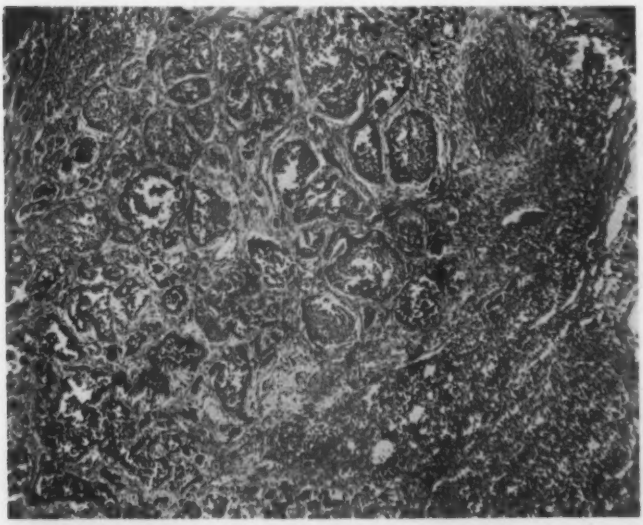

Fig. 4.—Carcinoid metastatic to mesenteric lymph nodes; mag. × 50.

abnormality consisted of a firm, circular nodule, 6 mm. from the tip of the ulcer. The tumor was 8 mm. in diameter and 2-3 mm. in height and appeared to be covered with normal intestinal mucosa. The cut surface disclosed uniformly firm tan tissue. The mesenteric lymph nodes were enlarged up to 2 cm. in diameter. They were discrete, tan, and firm. Histologically, this nodule (Fig. 3) and the mesenteric lymph nodes (Fig. 4) contained a carcinoid tumor. The tumor cells were in small nests and cords amid a highly vascular, loose connective-tissue stroma and were monotonous in their uniformity. The nuclei were central, had sharp nuclear membranes, and contained finely granular basophilic stippling. The cytoplasm was pale pink and vacuolated, with indistinct cell borders, so that the cytoplasm of adjoining cells appeared to blend with one another. The tumor tissue invaded the muscularis but did not reach the serosa. Carcinoid tissue infiltrated the muscularis mucosa and mucosa to form an ulceration coated with fibrin, neutrophils, and necrotic tumor tissue. Mitotic figures, in the primary and metastatic sites, were rare. Fontana-Masson stains[5] for argentaffin granules were performed upon tissues from the primary and secondary lesions. Argentaffin granules were found.

Comment

There have been 16 previously reported cases of carcinoid arising in a Meckel's diverticulum (Table). Meckel's diverticulum is found routinely in 2%-2.5% of all autopsies, with incidence in males predominating in a ratio of 2:1 or 3:1.[20,21] This may account for the disproportionate frequency of carcinoid of Meckel's diverticulum in men (15 of 17 cases). Of the 17 cases, 9 were discovered in the sixth decade of life; only 1 case occurred below the age of 25,[6] and its authenticity is doubted by Steward and Taylor,[7] who reviewed the original slides. Nine of the tumors were incidental findings (i.e., asymptomatic) at necropsy or laparotomy; six were associated with abdominal pain or acute diverticulitis; one tumor was found at laparotomy for suspected carcinoma of the colon with liver metastasis,[16] and the carcinoid tumor in our case was associated with intestinal hemorrhage either from a simple ulcer or from a carcinoid tumor in Meckel's diverticulum. The ulceration was distinctly separate from the carcinoid tumor, and, histologically, the ulcer contained no carcinoid tumor or aberrant gastric or pancreatic tissue.

The largest tumor in this series was the size of a "small orange,"[9] whereas the others were between 3[8,13,19] and 15 mm.[16] (pea size[7]) in diameter. Only 3 of the 17 cases had metastatic foci: Case 12[15] had massive liver metastasis; Case 13[15] had a solitary 5 mm. hepatic nodule (an incidental necropsy finding), and in our case (Case 17) the growth had spread to the regional mesenteric lymph nodes. None of these three patients had the clinical features of "carcinoid syndrome." Special stains for argentaffin granules were positive in 8 of 11 cases, and argentaffin stains were not obtained in the 6 remaining cases.

Summary

Carcinoid tumor arising in Meckel's diverticulum is uncommon. A case is presented, and the 17 reported cases are reviewed.

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Chronic Interstitial Pneumonitis

A Histogenetic Study

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No clearly defined concept of the basic histogenetic processes of chronic interstitial pneumonitis exists. This has come about partly because opinion regarding the origin of the alveolar macrophage is divided; because knowledge of the structure of the alveolar wall, until recently, has been based solely upon observations with the light microscope, and because the nature and potential of the alveolar lining cell are poorly understood. Nevertheless, the need for a precise delineation of the fundamental reactions in chronic interstitial pneumonitis is manifest in order better to understand the many pulmonary diseases with vastly different etiologic backgrounds which fall in the general category of interstitial pneumonitis. These diseases include all of the pneumoconioses, the pulmonary infections caused by Mycobacterium tuberculosis, by some fungi, and by some viruses, and inflammations of obscure etiology, such as sarcoidosis and Hamman-Rich disease, as well as others.

The purpose of this paper is to describe observations made on animal and human material which seem to indicate that certain fundamental processes within and upon the alveolar wall are common to the various structural modifications characteristic of different types of interstitial pneumonitis.

Materials and Methods

Most of the animal lungs stemmed from an investigation* in which 240 tuberculin-positive guinea pigs in groups of 15 or 30 were given single or multiple intratracheal injections of iron-contaminated quartz dust in aqueous suspension, either alone or in combination with one of the following: (*a*) PPD tuberculin, 0.005 mg.; (*b*) living tubercle bacilli (human, low virulence); (*c*) formalin-killed tubercle bacilli (same strain as above). Each of the three above additives was injected intratracheally alone into additional groups of sensitized guinea pigs as controls.

Other animal lungs were derived from experiments in which sensitized guinea pigs were exposed in an inhalation chamber for one year to a dust composed of atomized skimmed milk. There were also lungs from rats that had survived an intratracheal injection of quartz dust for different time periods.

Some of the animals died of intercurrent disease, and some, of the experimentally induced disease. Other animals were killed with ether during the course of the investigation at various intervals, and the survivors were similarly killed at the end of 12 to 13 months.

The lungs were fixed in formalin. Most animal lungs were distended with formalin under a 10 cm. head of pressure, but some were inflated with air. Following paraffin impregnation, sections were cut at 6μ.

The human material was varied: There were three cases of asbestosis, one of which was pure, whereas the other two were mixed with silicosis; there was one case of berylliosis; one of bauxite pneumoconiosis (Shaver's disease); one of Hamman-Rich disease; two of tuberculous pneumonia, and three cases of interstitial pneumonitis of undetermined character discovered as incidental findings during routine postmortem examinations.

All lung sections were stained routinely with hematoxylin and eosin, and some replicate sections were stained with Van Gieson's method. Interesting fields were photographed on 2¼×2¼ in. (5.7×5.7 cm.) roll film. The relative positions of the fields were noted by means of a Field Finder† and recorded. The sections were then decolorized and subjected to the silver-impregnation technique of Gordon and Sweet for the demonstration of reticulin fibers. The same fields were then re-

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†Sold by W. & L. E. Gurley, Engineering Instruments, Troy, N.Y.

photographed at the same magnification. Sections which contained silica were incinerated at 600 C, treated with concentrated HCl, washed, and the same fields were then photographed for the third time, but under dark-field conditions. Altogether, 1,330 fields were studied, involving about 3,400 photomicrographs.

Observations

Experimental Animals.—The early lesion is generally focal and involves the alveolar walls near a blood vessel. It consists of thickening of these walls by an increased number of cells. The boundaries of the cells within the alveolar wall are so poorly defined in the routinely stained sections that their relation to each other and to other alveolar structures is difficult to ascertain. The nuclei are round or oval and of two types: vesicular, resembling those within alveolar lining cells, and slightly smaller, darker ones, resembling lymphocytic nuclei (Fig. 1). Lesions are seen where the lymphocytic-type cell predominates and where the latter may be so numerous as to resemble a leukemic infiltration. More frequently, it is the alveolar-type cell that is, by far, the most abundant.

The reticulin-fiber structure of the thickened walls is quite variable. In the early lesions, perpendicular branches extend from the axial fiber and course between cells, tending to surround them. Less frequently the alveolar axial reticulin is irregularly thickened without significant branching. In more advanced stages both types of reticulin changes occur, associated with elongation and further arborescence of the branch fibers to form a complicated network that seriously compromises the alveolar lumen (Fig. 2).

The thickness of the alveolar wall may vary from twice normal to the diameter of the alveolar space. In the latter instance, of course, the space is obliterated. Although the growth of reticulin fibers generally keeps pace with the increase in the wall thickness, exceptions to this are not rare. Within the alveolus, the growing network of reticulin fibers lacks delimitation and may terminate in the obliteration of the air

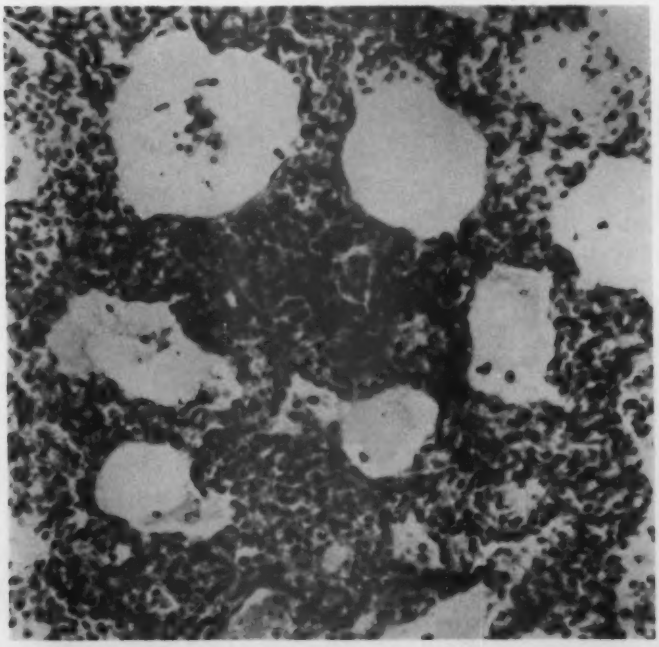

Fig. 1.—Guinea Pig 591, given 150 mg. of iron-contaminated quartz dust intratracheally in divided doses, died eight months following the initial dose. This interstitial pneumonia with cellular, greatly thickened septal walls shows a number of large, smooth-walled air spaces and some smaller, poorly outlined alveoli. The larger air spaces are not alveoli but alveolar ducts. Hematoxylin and eosin stain; reduced to 88% of mag. × 208.

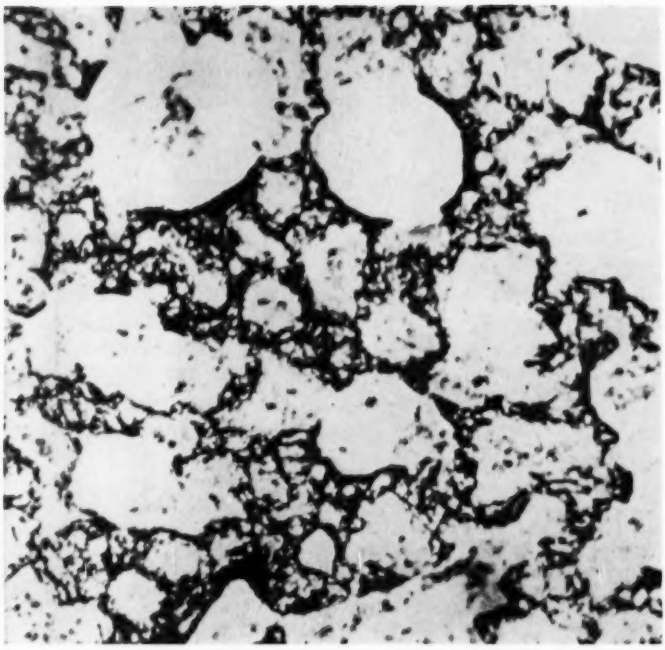

Fig. 2.—Same field as in Figure 1. Alveoli that were only vaguely indicated, as well as other alveoli which were not discernible in Figure 1, are now clearly outlined. The reticulin fibers form complex, irregular networks of variable thickness which extend into the air spaces without delimitation, narrowing some and obliterating others. This is characteristic of the accretive type of interstitial pneumonitis. Gordon and Sweet's silver-impregnation method; reduced to 88% of mag. × 208.

Fig. 3.—Guinea Pig 413 was given a single dose of 50 mg. of iron-contaminated quartz dust with 0.005 mg. PPD tuberculin intratracheally, and died 10 months later. This is part of a circumscribed mass of cellular solidification in which most alveoli are obliterated, although a few scattered, small, irregular spaces are present. Most of the nuclei seem to lie in a matrix. Only few cells are clearly or partially outlined. Hematoxylin and eosin; reduced to 92% of mag. × 208.

space (Figs. 2 and 4). In some lungs, the broad network of reticulin fibers may be delimited by a relatively thick band or strand of fibers which outlines a moderately large, ovoid or round air space (Fig. 2). Usually such an air space is not an alveolus but an alveolar duct which has been converted into a simple tubular structure by the obliteration of its evaginating alveoli.

In those instances where the growth of reticulin fibers has lagged behind the proliferation of alveolar cells, the alveolar wall as a whole is significantly broader than its reticulin framework. Where the periphery of the alveolar wall appears to extend significantly beyond the limits of its reticulin framework, the question may arise whether the cells peripheral to the reticulin are truly an integral part of the alveolar wall or are merely desquamated cells in close apposition with the latter. This question is more pressing when alveolar cell masses obliterate multiple air spaces to form solid nodules (Fig. 3). The occurrence of clefts does not necessarily prove that the cells peripheral to the fissure have desquamated, since the latter could very easily be artifactual. There are several criteria which are helpful in answering this question.

Desquamated alveolar cells, though crowded, generally show well-defined cell boundaries; the nuclei of the cells are round, and the character of the cytoplasm may vary from cell to cell. If the intra-alveolar cells constitute a tissue integral with the alveolar wall, the cell boundaries are generally indistinct; the nuclei are ovoid, and the cytoplasm is homogeneous, resembling a matrix (Fig. 3).

An interstitial pneumonitis may be represented by only a few widely scattered, small foci of septal wall thickening in which the air spaces are but little compromised. Obversely, the severity and extensiveness of involvement may be such as to seriously reduce the functional capacity of the lung. In some lungs, extensive carnification involves major portions of several lobes and is composed of closely studded nodules or more irregular foci up to several millimeters in diameter. In these nodules or foci, the alveolar architecture is obliterated by

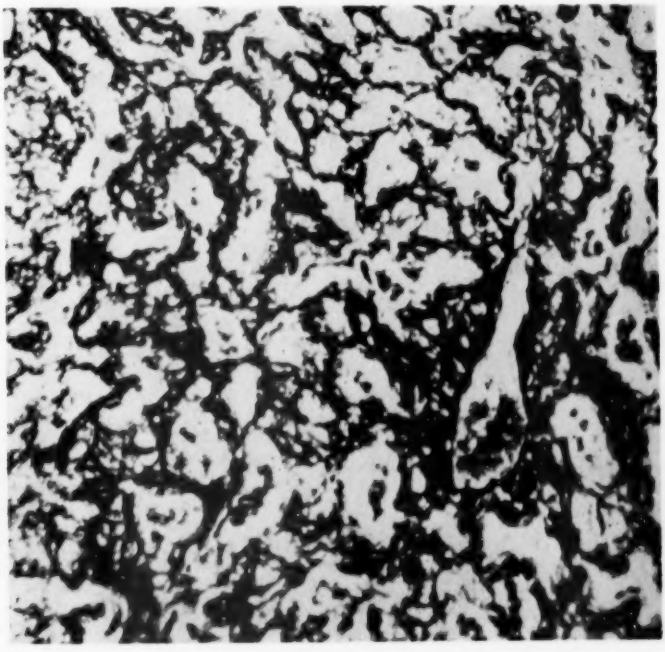

Fig. 4.—Same field as in Figure 3. The alveolar structure is surprisingly well preserved by irregularly thickened, partially collagenized walls from which very fine networks of reticulin fibers extend into the alveolar lumens. This, with the associated proliferated alveolar cells, results in varying degrees of partial alveolar obliteration and represents a more advanced stage of the accretive type of interstitial pneumonitis. Gordon and Sweet's silver-impregnation method; reduced to 88% of mag. × 208.

crowded cell masses and their reticulin fiber network. Scattered small remnants of air spaces may remain patent in the otherwise solid tissue (Fig. 3). It is common to find that silver impregnation demonstrates a much better preservation of alveolar structure than might appear to be the case in the routinely stained preparation. This is usually so because some of the seemingly obliterated air spaces are merely temporarily plugged by desquamated cells which are unaccompanied by reticulin (Fig. 1).

The alveolar capillaries are usually difficult to identify in the involved tissue, and evidence of exudation is generally lacking except for the presence of scattered eosinophils in some lungs. As a matter of fact, it almost appears as though an acute exudative inflammation is incompatible with a chronic interstitial pneumonitis, at least so far as the reticulin component is concerned. In the presence of a superimposed infiltration of neutrophilic polymorphonuclear leukocytes the finer, newly formed intra-alveolar reticulin network tends to disappear (enzymatic digestion!), so that, instead of alveolar obliteration, there may be a striking accentuation of alveolar structure by the thickened axial alveolar reticulin that remains relatively unaffected.

It is frequently very difficult to differentiate complete atelectasis from interstitial pneumonitis. This can be done with certainty only by studying the reticulin-fiber structure of the tissue. In severe atelectasis, alveolar reticulin is arranged in parallel layers, without, however, any communicating fibers between the layers. In interstitial pneumonitis, although partial atelectasis may be a prominent component of the lesion, it is characteristic of most interstitial pneumonias that there are communicating reticulin fibers which connect apposed alveolar walls.

As the lesions mature, the cellularity of the widened walls diminishes; the outlines of the surviving cells become discernible; the borders of patent air spaces become smoother and more sharply defined, and collagen fibers become abundant, while the

Fig. 5.—Case of asbestosis mixed with silicosis. Death from tuberculous pneumonia. Cellular thickening of alveolar walls with angioma-like capillary proliferation. The loose network of reticulin fibers comprising the framework of the thickened alveolar walls is characteristic of the acretive type of interstitial pneumonitis. The very numerous, large capillaries are clearly outlined. Gordon and Sweet's silver-impregnation method; reduced to 92% of mag. × 208.

complex network of fine reticulin fibers is largely replaced by a smaller number of thicker, more-wavy-than-looped, parallel fibers.

Capillaries which were difficult to identify in the earlier lesions are no less difficult to study in the later lesions. Information regarding the part played by vessels in the evolution of interstitial pneumonitis is therefore lacking.

Human Studies.—Lesions in human material as early as some of those in experimental animals are not found. However, lesions corresponding to a relatively early prefibrotic stage are found in a case of florid asbestosis, one of berylliosis, and two cases of tuberculous pneumonia. Here the alveolar walls are greatly widened by a fine reticulin network enmeshing alveolar cells and scattered lymphocytes. Plasma cells are also seen in the mural thickening due to beryllium. In older lesions of other cases the septal thickening is much less severe, less cellular, more collagenous, and quite dense. The collagenous components may be irregularly distributed in the thickened walls (Fig. 6). Alveolar obliteration is not threatened here, particularly since these air spaces are greatly enlarged.

The reticulin framework of the alveolar wall is frequently not widened as much as the entire wall seems to be in the routinely stained sections. This is usually due to metaplasia or simple swelling of the surface epithelium, which projects beyond the limits of the framework. This framework has a highly variable structure, and in moderately thickened walls there are irregularly thickened segments of reticulin bordered on either or both sides by a single layer of loops or arches of finer fibers. In regions of more pronounced septal thickening, there are multiple layers of such arched fibers or loops that surround the proliferated alveolar cells (Fig. 5). Combinations of these two patterns are very common.

The development of collagen in the thickened septal walls is frequently associated

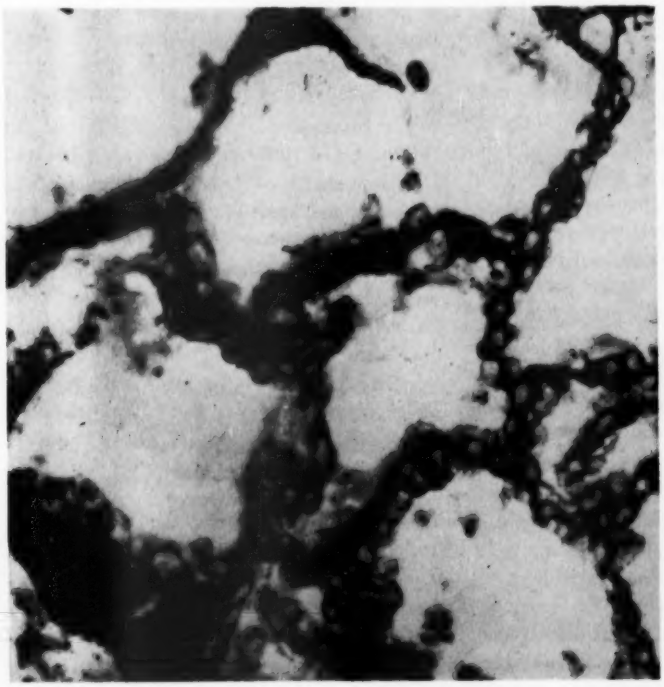

Fig. 6.—A case of asbestosis showing the uniformly thick collagen illustrative of the expansive type of interstitial pneumonitis. An angioma-like proliferation of capillaries is also evident here. Gordon and Sweet's silver-impregnation method; reduced to 92% of mag. × 208.

with central segmental thickening of the reticulin framework or in the replacement of the arched or loop-like pattern by coarser, more or less parallel fibers, or both. Coincidental with the collagenization, the cellularity of the wall becomes greatly reduced.

In a case of tuberculous pneumonia, besides an interstitial involvement corresponding to the prefibrotic stage, there are also regions in which the alveolar reticulin framework is focally attenuated, disrupted, and fragmented, even though overt necrosis is not present. No reticulin is found in regions of caseous necrosis.

Alveolar capillaries are very prominent in some of the human material. This is particularly true in the cases of asbestosis where, in many regions, a profusion of thin-walled, distended alveolar capillaries is almost angioma-like (Figs. 5 and 6). These capillaries are usually to be found upon only one surface of a given length of a thickened, collagenous septal wall, but alternation of surfaces upon which the capillaries occur and a certain amount of overlap are common enough. Thick, collagenous septal walls are therefore seen where thin-walled capillaries are present upon both surfaces in apposed position. An interesting feature of this vascular arrangement is the fact that not only are most of the capillaries excessively large and have a major portion of their circumference presenting to the alveolar lumen but the free portions of the capillary circumference usually demonstrate no significant thickening of the wall which could constitute an alveolocapillary barrier (Figs. 5 and 6). There are regions in asbestotic lungs where an occasional capillary is found embedded in collagen, but much oftener the densely collagenized wall is found to be avascular.

Comment

In interstitial pneumonitis, alveolar walls are thickened, owing to the reaction of its component tissues to injury. In bronchopneumonia or lobar pneumonia, the alveolar wall is not known to undergo significant structural change other than the distention of the alveolar capillaries, which are the source of the characteristic exudate in the air spaces.

An alveolar wall may be thickened by the expansion or the spreading apart of its lateral limits or by the addition of tissues upon its lateral limits. Although observations indicate that expansion of the alveolar wall occurs, the early stages of such expansion are not represented in the material studied, or they have not been recognized. Inasmuch as a space or a potential space has been demonstrated by electron photomicrography [1,2] to exist between the basement membrane of the capillary and the basement membrane of the alveolar surface epithelium, it is in this space that cells, fluid, and tissue fibers must accumulate to furnish the force necessary for the expansion.

Much commoner in the material at hand has been thickening of the alveolar wall by the addition of cells and fibers upon its surface. This accretive growth of cells (growth by addition to the external surface) forms an integral part of the alveolar wall and thereby differs from an exudate. The reticulin fibers give additional physical support to the unity of the excrescence with the wall.

The origin of the cells responsible for the increased thickness of the alveolar wall is a matter of conjecture, since the study material was static. It seems most reasonable to believe that the cells were descendants of existing alveolar cells, although it must be admitted that mitoses were not seen. Nevertheless, Bertalanffy and Leblond [3] have demonstrated, by counting mitotic figures in alveolar cells of rats, that the stock of vacuolated alveolar cells is renewed in about 29 days and the stock of nonvacuolated alveolar cells in about 8 days. The other view, that the cells are mononuclear leukocytes derived from the blood, seems less likely. It is interesting that Policard and Collet,[4] in their studies of early silicotic granulomata with the electron microscope, noted rupture and disappearance of the alveolar basement membrane, while the cells

upon the alveolar surface became joined inseparably with the alveolar wall. These investigators also noted the disappearance of the alveolar capillaries. The association of reticulin fibers with the appearance of multiple cell layers upon the alveolar surface and the syncytium-like character of the alveolar-cell mass also speak against an origin from leukocytes of the blood and for an origin from existing tissue cells.

Although most giant cells contain abundant dust, in several alveoli multinucleated cells which contain no phagocytosed material are found associated with many macrophages heavily burdened with phagocytosed particles. It is tempting to conclude from this association that the non-dust-containing giant cells were formed not by a fusion of desquamated macrophages but by the desquamation of a syncytial mass which had been preformed upon the alveolar wall. However, whereas the latter is a possibility, it would be premature to make this claim.

In regions where many alveoli show massive desquamation of alveolar cells, no reticulin-fiber branches protrude at right angles from the wall to mark the sites where the cells were at one time attached. This absence of reticulin-fiber branches could be interpreted either that such branches never developed or that the branches became resorbed. In either case, cells without reticulin-fiber support seem to be vulnerable to influences which cause desquamation. A known, common cause of resorption of reticulin fibers and alveolar desquamation is a superimposed acute exudative inflammation.[5]

The nature of many of the lymphocyte-type cells in the thickened septal walls is probably similar to that of the morphologically identical cells found in perivascular and paravascular cell collections of rats and guinea pigs. Cogent reasons have been advanced for believing the latter cells to be mesenchymal derivatives of multipotential alveolar cells.[6] Serious doubt exists that many cells morphologically identical with lymphocytes, and other, slightly different, yet intimately associated cells are actually lymphocytes, because of certain functional anomalies.[5] However, that some of these cells are indeed lymphocytes morphologically, as well as functionally, need not be questioned. It is also very probable that the plasma cells which are prominent in some forms of interstitial pneumonitis are derived by metaplasia from lymphocytes, as has been observed in lymph nodes.[7]

It appears improbable that expansion of the potential space between the capillary and the alveolar basement membrane could attain a dimension large enough to obliterate an alveolar space. On the other hand, obliteration of alveoli by accretive growth upon the alveolar surface is readily demonstrable in different types of interstitial pneumonitis, caused by a variety of etiologic agents.

Obliteration of alveoli tends to simplify the pulmonary structure so that alveolar ducts become converted into more or less smooth-walled cylinders, which, when seen in cross section, may give the mistaken impression of being enlarged alveoli. Interstitial inflammations of greater severity result in more complete destruction of the pulmonary architecture. The accretive growth of cells and fibers here continues with the involvement and final obliteration of alveolar ducts and respiratory bronchioles. The end-product of this process may be a discrete nodule or a more irregular fibrous lesion. Extensive fibrosis involving major portions of lobes results from confluence of such lesions. Necrosis is not a prerequisite of fibrosis, since collagen is demonstrable without necrosis, as well as in lesions which show only early necrosis.

Except where air spaces are lined by metaplastic cuboidal or columnar epithelium, it is reasonable to interpret any considerable extension of alveolar-wall thickness beyond the limits of its reticulin network as evidence of recent accretive growth and, therefore, of active inflammation.

Whether the alveolar-wall thickening occurs by expansion from within the wall or

by accretive growth upon the external surface may be important to the survival of the capillary vessels. It is likely that capillary obliteration would occur with an expansive deposition of collagen that envelops and ultimately constricts the vessel. One explanation which could be advanced for the observed mural vascularity in these asbestotic lungs is that the expansive deposition of collagen within the alveolar wall was asymmetric and occurred upon one side or the other of the capillary, thus merely displacing it without impairment of the lumen. However, this explanation would not serve to explain the presence of capillaries in apposed positions on *both* surfaces of the collagenous wall.

If one assumed that alveolar capillaries could proliferate and form a new network upon the surfaces of sclerotic walls, then the appearance as herein described would be adequately explained not only for the expansive type, but also for the accretive type, of mural thickening. Such an explanation has been offered by Pratt,[8] who believes that the administration of oxygen is the stimulus for the growth of these capillaries. The extent to which this treatment is a factor in the present cases is not known.

Summary

In interstitial pneumonitis, the alveolar wall becomes thickened in one, or possibly in both, of two ways:

1. By expansion of its lateral borders, due to pressure of fluid, cells, connective tissue fibers, or combinations of these in the space between the capillaries and the surface epithelium
2. By an accretive growth upon alveolar surfaces of alveolar cells and their associated reticulin network

Whereas the former gives rise to moderately thickened collagenous walls, it tends to leave alveolar spaces intact. The accretive growth of alveolar walls, on the other hand, compromises the alveolar spaces and often obliterates them. By the obliteration of contiguous air spaces, nodulations or more irregular foci of consolidation are formed, which ultimately may undergo collagenization and hyalinization. By confluence of these foci, major portions of lobes or entire lobes may become severely scarred and impaired in function.

Because capillaries are difficult to identify in the pulmonary lesions of experimental animals studied, information regarding the contributions which these vessels make to interstitial pneumonitis is lacking. Human material offers only fragmentary information on this point. On the basis of observations in two cases of asbestosis, it appears likely that expansive alveolar fibrosis may be asymmetrical; i.e., the deposition of collagen may occur on only one side of a capillary over a given length, thereby allowing partial respiratory function. By the same token, it is likely that a capillary will become obliterated if collagen is deposited along both sides of the capillary in the space or potential space situated between the basement membrane of the capillary and that of the alveolar surface epithelium. Evidence that alveolar capillaries may proliferate is seen in profusions of capillaries found in apposition on both surfaces of thick, sclerotic alveolar walls.

Conclusions

Two basic types of inflammatory reaction in alveolar walls are described. One or both of these reactions may be encountered in interstitial pneumonitis regardless of etiologic background.

In one, the alveolar fibrosis is caused by the deposition of collagen between capillary and the alveolar-covering epithelium and is therefore an expansive thickening.

In the other, the alveolar fibrosis is initiated by an alveolar-cell proliferation upon the alveolar surface. These proliferated cells form an integral part of the alveolar wall, and therefore the thickening is accretive.

Expansive fibrosis causes only moderate thickening of septal walls without significantly compromising the air spaces.

Accretive alveolar involvement may seriously compromise air spaces and obliterate them. Obliteration of contiguous air spaces by accretive alveolar thickening may lead to the formation of nodules or more irregular scars encountered in the various more deforming types of interstitial pneumonitis.

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Carcinogenic Studies on Soot of Coffee-Roasting Plants

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The present studies on soot from coffee-roasting plants have their origin in the observation of unusually high morbidity and mortality rates for cancers of the lung, larynx, and bladder reported by Warran,[1] in 1951, for the resident population of New Orleans. While similar rates for cancers of the lung have been observed in other American cities of industrialized type, such as Philadelphia, Chicago, and San Francisco,[2] they are rather surprising for a metropolitan community of mainly commercial character, such as New Orleans (Table 1). Because of this special aspect of the findings from New Orleans, it was felt that an analysis of the environmental conditions which exist in and are peculiar to this city might yield information pertinent to the cause or causes of the reported phenomenon, since they are less complex than those encountered in industrialized communities.

Considering the sites of the three cancers with an excessive morbidity rate, it was likely that the causal factor, or factors, might be present either as air pollutants or as constituents of foodstuffs. A survey of the various industrial activities carried on at New Orleans showed that effluents from numerous coffee-roasting plants in the city made a noticeable and unusual contribution to air pollution and that some of the coffee beans roasted were subjected, according to local customs, to a severe degree of roasting, causing a superficial charring of the beans. Soot from the smokestacks of two coffee-roasting plants, one using the direct method of roasting, in which the flame is in contact with the beans, the other employing an indirect method, were obtained through the courtesy of the management of these plants and by the cooperation of the Bureau of Industrial Hygiene, Louisiana State Health Department, New Orleans (Mr. W. H. Reinhart), and of the Sanitary Engineering Center, U.S. Public Health Service, Cincinnati (Dr. L. Chambers).

The results of a chemical study of this soot for the presence of carcinogenic hydrocarbons, which were published sometime ago,[3] demonstrated the presence of 3,4-benzpyrene in both types of soot. The present communication deals with the bioassays

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TABLE 1.—*Cancer Incidence (I), Prevalence (II), and Mortality (III) Rates per 100,000 Population* *

	1947								
	Lung			Larynx			Bladder		
City	I	II	III	I	II	III	I	II	III
New Orleans	20.8	24.0	13.6	7.6	11.1	3.6	19.1	24.2	5.2
Atlanta	8.9	10.8	6.9	2.0	3.1	0.6	6.0	8.9	3.5
Philadelphia	20.5	25.9	14.5	4.8	6.7	1.8	14.4	21.3	6.4
Chicago	18.0	20.7	14.2	3.7	5.7	2.3	12.7	16.3	5.8
Pittsburgh	15.6	18.3	10.8	4.4	5.5	1.6	12.3	16.6	5.0
San Francisco	20.8	25.3	13.8	4.6	6.4	1.6	12.8	17.6	5.6

* Prepared from data contained in issues No. 1, 2, 3, 5, 6, and 10 of Cancer Morbidity Series, published 1950, 1951, and 1952 by U.S. Public Health Service, Federal Security Agency, Washington, D.C.

of these soots for carcinogenic properties for mice, rats, and guinea pigs when administered by cutaneous application, intramuscular introduction, and inhalation.

Experimental Procedures

The coffee soots whose technologic aspects and characteristics were described in the previous publication[8] were ground to a very fine powder in a micropulverizer before they were processed and used. For this purpose the micropulverizer was cooled with carbon dioxide snow (Dry Ice), thereby making the normally somewhat oily and sticky soot hard and brittle, and permitting the grinding process to proceed without clogging the pulverizer with glue-like masses of soot.

The following procedures were followed in the four experiments used in the testing of the coffee-roast soot for carcinogenic properties.

1. *Intramuscular Injection of the Residue of the Acetone Extract of Coffee-Roast Soot.*—In the preparation of the residue of the acetone extract of coffee-roast soot, 10 gm. of the soot recovered from direct and indirect roasting processes were extracted in a Soxhlet apparatus with 100 cc. of acetone. The acetone extract was condensed by the evaporation of the acetone. A solid, dark-brown fudge-like material with some tan-and-white-colored particles remained. This residue was dissolved in tricaprylin. Since the subcutaneous injection of a dilution of 1,000 mg. of residue in 1 cc. of tricaprylin caused the death of all mice when 0.05 cc. of this material was administered, the concentration of the residue was reduced to one-fourth of the originally chosen one, i.e., 250 mg. of the residue in 1 cc. of tricaprylin. Six injections of 0.05 cc. each of this material were made in biweekly intervals into the right thigh muscle of mice. Residues of the two types of soots were tested separately. Fifty mice of the C57 black strain equally divided as to the two sexes were used for each type of residue. Sets of 30 rats (Bethesda black strain, females 3 months old) for each type of residue received 12 injections of 0.2 cc. of the suspension of 500 mg. of residue in 1.0 cc. of tricaprylin into the right thigh muscle at monthly intervals.

The maximal observation period was 24 months, at the end of which all surviving animals were killed. All animals were studied at autopsy, and their organs when showing any pathologic lesions of possible significance were examined histologically.

2. *Repeated Cutaneous Administration of Residue of Acetone Extract of Soot.*—Ten gm. of the residue of the acetone extract of the soot was suspended in 25 cc. of acetone by grinding the mixture in a mortar. Upon standing, a dark-brown-colored clear fluid formed above a brownish-white sediment. The preparation had to be well shaken before application to the nape of the neck. Paintings were performed twice a week to 50 C57 black mice (equally divided as to sex (Strain 6)) for a maximal period of 24 months.

3. *Intramuscular Implantation of Coffee Soot Suspended in Sheep Fat.*—For the implantation of coffee soot, pellets consisting of coffee soot obtained by the indirect method of roasting mixed with sheep fat produced by the extraction of fresh sheep fat with benzene in a Soxhlet apparatus were used. A few drops of acetone were added to 1.25 gm. of soot and 3 gm. of fat for facilitating the mixing process. The mixture was shaped into a cube, refrigerated to hardness, and then cut into five equal pieces, each containing approximately 250 mg. of the soot. These pellets were implanted by surgical procedure into the right femoral muscle of 30 rats (Bethesda black, females, 3 months old). The maximal observation period was 24 months.

4. *Inhalation of Finely Powdered Coffee Soot.*—During the first seven months of the inhalation experiment the micropulverized soot was delivered into the dusting chambers to maintain a concentration of about 15 mg. per cubic meter of air for five hours a day, four days a week, using a Wright Dust Feed Mechanism. It appeared advisable to intensify considerably the exposure of the rats and guinea pigs to soot because it contained doubtlessly a large amount of carcinogenically inert matter. A heavy-walled glass flask of 1 liter capacity was filled with 250 gm. of powdered soot. The soot in the flask was agitated by an electrically operated hammer, hitting the outside wall of the flask, as well as by the introduction of a stream of air into the bottom of the flask through a glass tube having a perforated bulb at its end. The cloud of dust thereby created was introduced under the pressure of the air stream through a plastic tube into the upper part of the dusting chamber. By this procedure it was possible to discharge daily from 150 to 400 gm. of soot into the chamber, increasing thereby the concentration of the soot to about 225 to 600 mg. of soot per cubic meter of air. Although the exposure to soot by inhalation, ingestion, and skin contact became thereby rather heavy, it was well tolerated.

A total of 60 rats of the Bethesda black strain (equally divided as to sex) and 25 guinea pigs (Strain 13) were kept in the chamber in eight separate cages. The maximal exposure period was 24 months.

Results

The neoplastic reactions and other significant lesions observed in the four experimental series are summarized in Table 2.

TABLE 2.—*Reactions in Mice and Rats After Cutaneous Application, Intramuscular Injection, or Inhalation of Coffee Soot and Residue of Acetone Extract of Coffee Soot*

Material and Route of Administration	Species	No. of Animals	Survivors Over 1 Yr.	Tumors	Other Lesions
Direct soot; acetone residue; painting	Mouse	50	36	1 lymphoma	Epidermal hyperplasia
Indirect soot; acetone residue; painting	Mouse	50	40	1 leukemia, lymphoid	Epidermal hyperplasia
Direct soot; acetone residue; intramuscular	Mouse	50	35	2 lymphomas 1 fibrosarcoma (thigh)	
Indirect soot; acetone residue; intramuscular	Mouse	50	3	--	
Direct soot; acetone residue; intramuscular	Rat	30	20	1 squamous-cell carcinoma, uterus 2 adenofibromas, breast 1 cholangioma	Multicystic, foreign-body, foam-cell granulomas at site
Indirect soot; acetone residue; intramuscular	Rat	30	24	1 anaplastic carcinoma, stomach 1 fibrosarcoma, thigh 2 adenocarcinoma, uterus 3 adenofibromas, breast	1 callus gastric ulcer in glandular mucosa
Indirect soot; intramuscular	Rat	30	22	1 round-cell sarcoma, ileocecal nodes 2 reticulum-cell sarcomas, liver 1 cholangioma	Multicystic foam-cell and giant-cell granulomas in thigh
Coffee roast soot; direct and indirect inhalation	Rat	60	51	3 adenomatosis, lung 8 reticulum-cell sarcoma, liver 1 squamous-cell carcinoma, skin (head) 1 adenocarcinoma, uterus 2 cholangiomas 1 cystadenoma, ovary 4 adenofibroma, breast	1 purulent chronic cystitis, ulcerative 3 calcifications of elastic tissue, lung 1 glandular hyperplasia, uterus
Coffee roast soot; direct and indirect inhalation	Guinea pig	25	25	2 adenomatosis, lung 2 carcinomas, bladder 1 papilloma, bladder	6 bronchiolar epithelial hyperplasia 2 calcification of elastic tissue, lung 1 calcification muscularis, stomach 4 cystitis cystica

Comment

A critical analysis of the various neoplastic and non-neoplastic lesions observed in the different series indicates that causal relations to the exposure to coffee soot or its acetone extract residue definitely exist for only some of these manifestations. For others such associations are of uncertain nature, while they can be excluded for a third group of lesions.

The mild to moderate epidermal hyperplasias of the skin of mice painted or intramuscularly injected with residue of acetone extracts of coffee soot are attributable to chemicals present in the soot. It is doubtful, on the other hand, whether the four lymphomatous reactions observed among a total of 200 mice composing the four series represent effects of the coffee soot residue applied to the skin of these animals. While such remote effects of cutaneously or subcutaneously administered carcinogenic hydrocarbons on the development of lymphomatous manifestations in mice have been reported,[4]

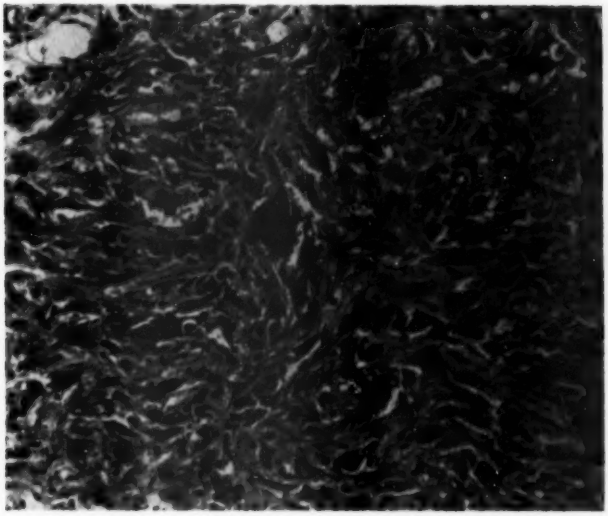

Fig. 1.—Fibrosarcoma of the thigh in a mouse given an intramuscular injection of the residue of acetone extract of soot from a coffee-roasting plant. × 205.

the number of lymphomatous animals seen in this experiment is rather low and is within the range of lymphomatous reactions reported to occur spontaneously in this particular strain. In a control series of 350 mice, 150 of which received subcutaneous injections of tricaprylin, only one lymphomatous animal was found. The fibrosarcoma of the right thigh observed in one mouse injected with the residue of acetone extract of soot from indirectly roasted coffee and measuring 2 cm. in diameter, on the other hand, represents a carcinogenic reaction attributable to the soot (Fig. 1).

An identical connotation must be given to the infiltratively growing fibrosarcoma which developed in the right thigh of one of the rats given intramuscular injections of residue of acetone extract of soot, or of soot in sheep fat. It is also most likely that the chronic callus ulcer of the glandular gastric mucosa seen in another rat, and showing glandular formations within fibrohyaline tissue, as well as the anaplastic

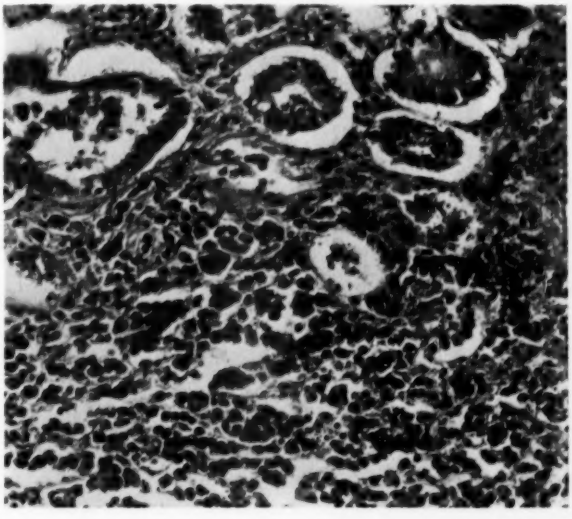

Fig. 2.—Anaplastic carcinoma of the glandular mucosa of the stomach of a rat given an intramuscular injection into the thigh of the residue of acetone extract of coffee soot. × 205.

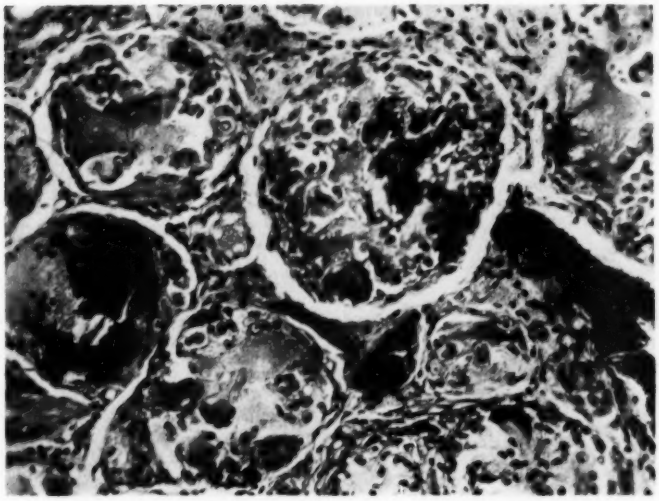

Fig. 3.—Giant-cell and foam-cell granuloma with intracellular and extracellular deposits of soot in the thigh of a rat. × 205.

carcinoma involving the entire gastric wall of a third rat (Fig. 2) of this series, represent reactions to chemicals present in the soot, especially since it is known that caffeine (present in up to 15% in the coffee soot used [3]) when injected intramuscularly into rats and guinea pigs, elicits peptic ulcers.[5] The other benign and malignant neoplasms found in rats of these three series, and affecting the breast, liver, lymph nodes, and uterus, have evidently no relation to the soot administered. Similar tumors are known to occur on a "spontaneous" basis in members of this strain.[6] The large multicystic foam-cell and giant-cell granulomas, with their massive amorphous brown intracellular and extracellular deposits, are non-neoplastic reactions products to the test material injected into the thigh (Fig. 3).

The pathologic manifestations noted in the rats and guinea pigs inhaling coffee soot are of special interest, since they are found in association with an exposure which is identical with that encountered by man. Again, many of the various benign and

Fig. 4.—Cylindrical-cell alveolar adenomatosis in the lung of a rat exposed to coffee soot by inhalation. × 205.

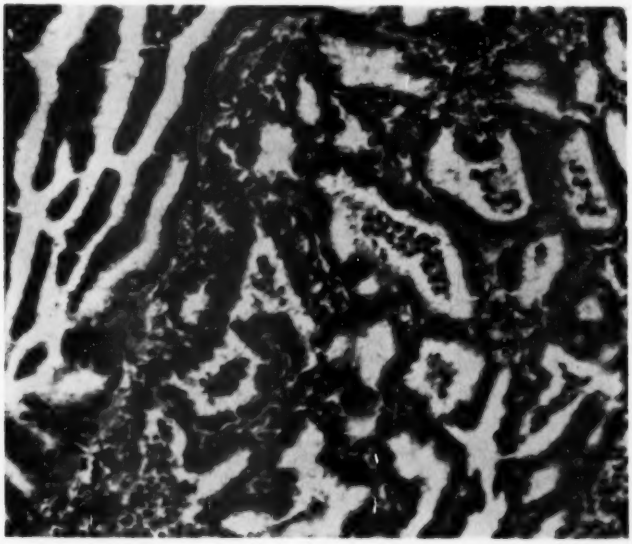

Fig. 5.—Alveolar adenomatosis in the lung of a guinea pig inhaling finely powdered coffee soot. × 205.

malignant tumors of the liver (reticulum-cell sarcomas, cholangiomas), uterus (carcinoma and endometrial glandular hyperplasia), ovary (cystadenoma), and breast (adenofibroma) found in rats can be considered as causally unrelated to the exposure sustained, and of "spontaneous" nature. The other pathologic reactions observed, however, most likely have such associations.

The extensive adenomatosis of the lung found in some rats and guinea pigs and characterized by glandular areas lined by a high cylindrical epithelium in the presence, as well as in the absence, of chronic inflammatory changes in this organ belong to the group of lesions with presumptive causal correlations (Figs. 4 and 5). Such alveolar changes may occasionally assume malignant character.[7,8] They are evidently in both rats and guinea pigs of polyetiologic nature and of relatively common occurrence and do not seem to possess "precancerous"

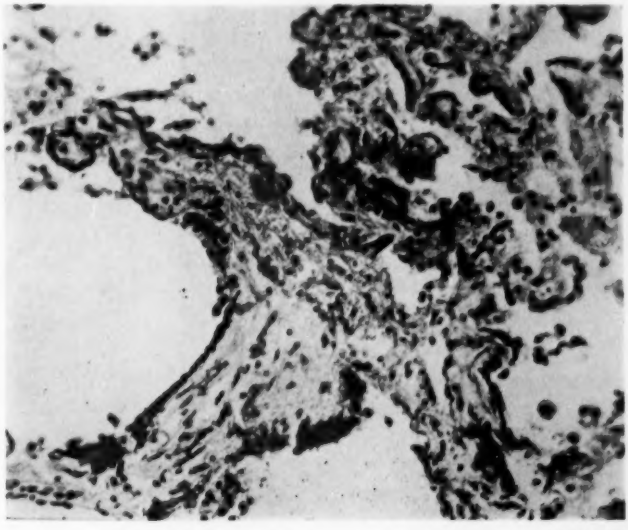

Fig. 6.—Thickened interalveolar septa with fragmented and calcified elastic fibrils in the lung of a guinea pig inhaling coffee soot. × 205.

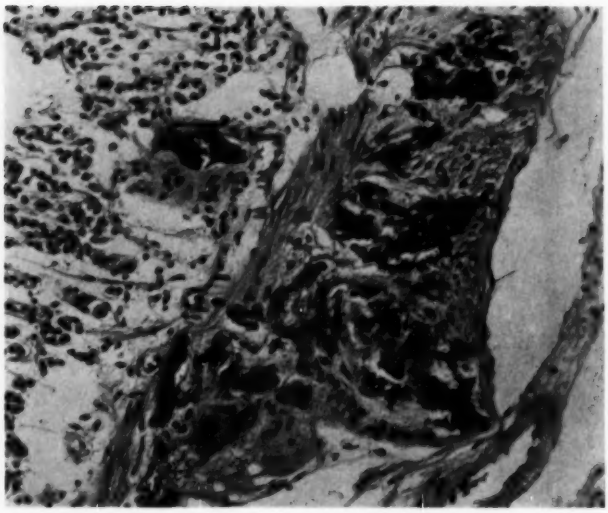

Fig. 7.—Massive focal calcification of the muscularis and glandular mucosa of the stomach of a guinea pig following inhalation of coffee soot. × 205.

properties, because "spontaneous" cancers of the lung are rarely observed in rats and guinea pigs under laboratory conditions.

The extensive calcifications and fragmentations of the elastic fibrils in the interalveolar septa of the lung and of the muscle tissue in the wall of the stomach of several guinea pigs and rats may represent the results of an acidotic state produced by the gastric secretion-stimulating effect of ingested or inhaled caffeine. These metastatic calcifications resemble in many respects those seen in man and animals following an administration or hypersecretion of parathyroid hormone, as the result of hypervitaminosis D and of destruction of bone or of excessive mobilization of calcium from the bones.[9]

One of the rats had a bean-sized node located at the base of the ear, which was histologically a warty, cornified squamous-cell carcinoma apparently elicited through

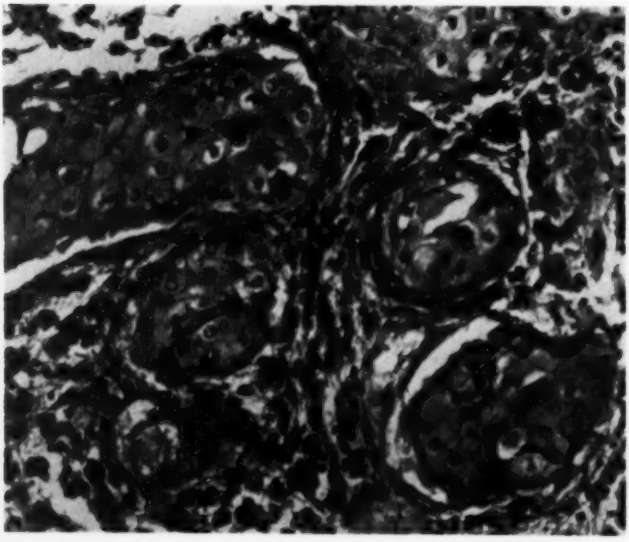

Fig. 8.—Small-cell anaplastic carcinoma of the mucosa of the bladder surrounding epithelial nests of a guinea pig after inhalation and ingestion of coffee soot. × 205.

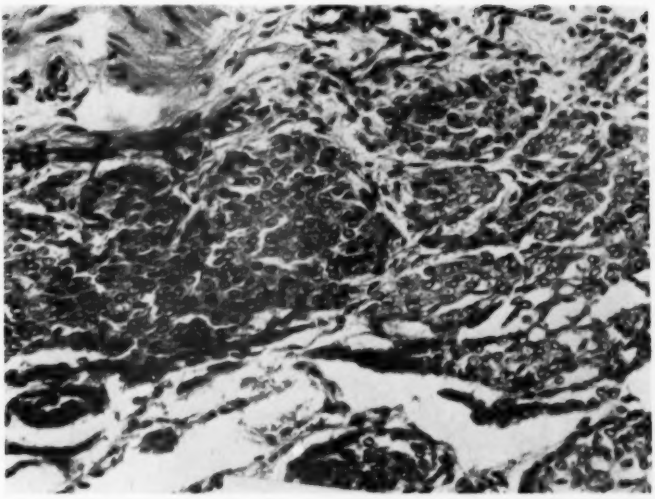

Fig. 9.—Nests of transitional-epithelial cells invading the submucosa of the bladder of a guinea pig which inhaled and ingested coffee soot. × 205.

the action of carcinogenic matter in the dust coming in contact with the skin. An unexpected finding was the demonstration of precancerous and benign and malignant neoplastic lesions in the urinary bladders in 7 of 11 guinea pigs which came to autopsy during the last six months of the experimental period. In one of these animals, a pea-sized, firm nodule was present attached to the dome of the bladder. It had a smooth surface. In a second guinea pig, a small, localized thickening of the mucosa of the bladder was found. In a third one a large pinhead-sized papilloma of the mucosa was observed, while in four additional animals the histologic examination of the bladder revealed the existence of extensive, focal lesions of a cystitis cystica type, which in man is suspected to possess precancerous potentialities.[10]

The large nodular tumor of the urinary bladder proved to be an anaplastic carcinoma consisting of small, hyperchromatic cells invading the bladder wall in its entire

Fig. 10.—Transitional-cell papilloma of the bladder mucosa with several submucosally located glandular formations in a guinea pig exposed to coffee soot by inhalation and ingestion. × 105.

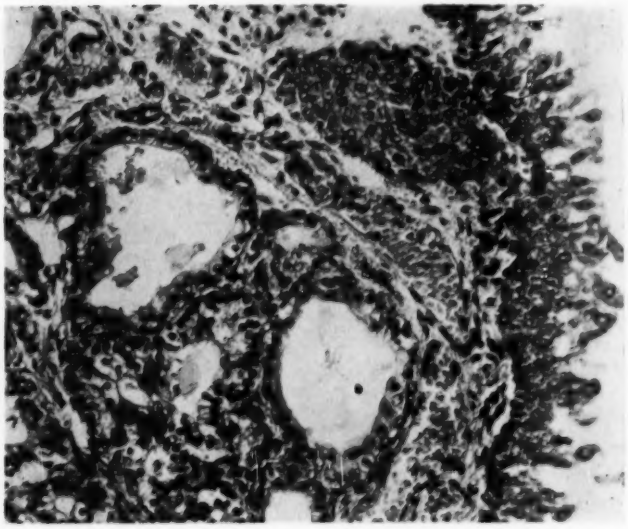

Fig. 11.—Beneath the transitional-cell lining of the bladder clusters of glandular formations and a solid transitional-cell nest are located in a guinea pig inhaling and ingesting coffee soot. × 205.

thickness and extending for some distance beneath the mucosa (Fig. 8). The malignant lesion present in a second guinea pig was represented by infiltratively growing nests of transitional epithelial cells invading the submucosa (Fig. 9). The papilloma present in a third guinea pig was a typical filiform transitional-cell fibroepithelioma, showing in one area a cluster of small glandular formations (Fig. 10). In the other four animals cystic lesions located beneath the epithelial lining were present at several places. These cysts were lined either by transitional epithelium or by cuboidal epithelium and represented manifestations of cystitis cystica (Figs. 11 and 12).

Considering the fact that tumors of the bladder in guinea pigs have not been observed before by other investigators [7,11] and were not found in animals of the same strain

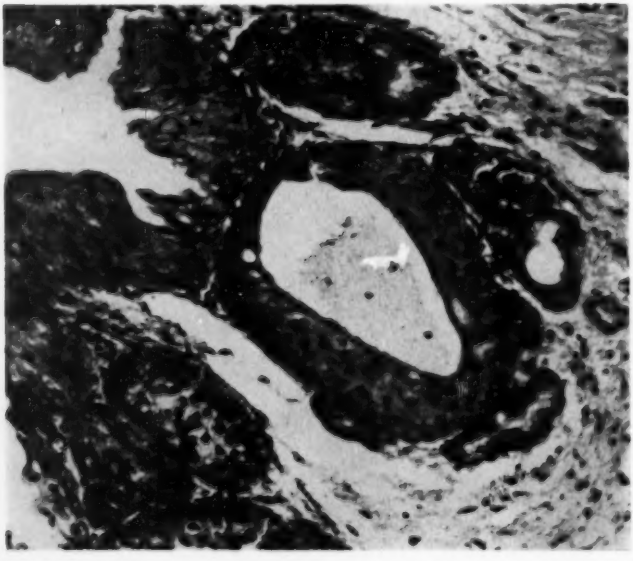

Fig. 12.—Cystic formations lined with transitional cells beneath the mucosa of the bladder in a guinea pig exposed to coffee soot by inhalation and ingestion. × 205

used in another experiment,[8] the conclusion seems to be justified that the precancerous, as well as the neoplastic, lesions observed in the bladder of guinea pigs inhaling and ingesting soot from coffee-roasting plants may causally be related to some substance or substances present in the soot and excreted in the urine. Such an effect appears to be more likely, since caffeine present in the soot is an excellent solubilizer of carcinogenic polycyclic aromatic hydrocarbons, has a diuretic effect, and is excreted in the urine. The basic prerequisites for a carcinogenic effect upon the bladder mucosa of specific substances presumably present in the coffee soot, therefore, existed.

The various observations made in mice, rats, and guinea pigs concerning the development of benign and malignant tumors at the sites of application, deposition, and excretion following an exposure to soot from coffee-roasting plants provide, in part conclusive, in part highly suggestive, evidence supporting a carcinogenic action of this air pollutant on several tissues of contact.

It is felt at present, however, that any assessment of the significance of these observations made in animals in relation to the high incidence rates of cancers of the lung, larynx, and bladder in New Orleans should be held in abeyance. From an inspection of 14 coffee-roasting plants located in this city for estimating the amount and local distribution of air pollutants originating from these sources, the conclusion was reached that a major portion of the respiratory cancers could not plausibly be attributed to the inhalation of soot from coffee-roasting plants. Similar considerations apply to the supposition of coffee soot being the main causal factor in the development of cancers of the bladder in New Orleans. Since recent studies on roasted coffee beans have demonstrated the presence of rather small amounts of 3,4-benzpyrene only in very dark roasted coffee of the so-called French type,[12] it is also not likely that even an excessive consumption of a drink prepared from ordinary roasted coffee plays a significant role in this respect.

Although there has been a progressive rise in the frequency of deaths from bladder cancer in several countries during the last decades (Denmark, England),[13,14] this can scarcely be related to an increase in the consumption of coffee, since the bladder cancer death rate is higher in England, where tea is the favored drink, than in the United States, which is one of the principal consumers of coffee. It is more likely that these developments are related to a growing use of certain food dyes used for the coloring of butter, margarine, and cheese, and cheese crackers, since it has been shown that these dyes contain a known and highly potent human carcinogen, namely, β-naphthylamine as an impurity,[15] which has been responsible for the occurrence of occupational bladder cancer in about 2,000 dye and rubber workers, necessitating the discontinuation of its industrial production in most Western countries (United States, England, Germany, and Switzerland).

A perhaps more direct connection with the problem on hand has been the observation of a relatively high incidence of bladder cancer in occupations associated with the handling of coal tar and pitch reported by Henry, Kennaway, and Kennaway in 1931,[16] since the development of these tumors is attributed by Williams [17] to the presence of very small amounts of β-naphthylamine demonstrated in such materials by Somerville and Williamson.[18] Whether or not this or other carcinogenic aromatic amines are formed during the roasting of coffee beans from an interaction of ammonia and aromatic compounds generated during this process from nitrogen-containing substances has as yet not been determined.

It is likewise uncertain whether the higher frequency rates of bladder cancer among urban residents over rural residents noted in several countries by Clemmesen [13] and Case [14] is associated with a severer exposure of the urban population to inhaled and ingested sooty materials containing aromatic compounds generated by the combustion of coal, oil, and gasoline and having possibly interacted with nitrous oxide.

Since the roasting of coffee beans involves the production of incomplete combustion products from vegetable matter, mention may also be made here of the reported positive statistical association between cigarette smoking and liability to bladder cancer recorded by Clemmesen,[19] Denoix and Schwartz,[20] and Lilienfeld et al.,[21] in Denmark, France and the United States. It is perhaps noteworthy that these associations were obtained in an American community in which a large dyestuff factory had created for many years a special occupational bladder cancer hazard which, however, was not demonstrated in the statistical analysis cited. The development of bladder tumors in mice following their intraoral painting with cigarette tar also has been reported.[22]

Despite a considerable amount of uncertainty concerning the significance of the various observations regarding the role of incomplete combustion products of different types of carbonaceous matter in the causation of bladder tumors in man, the total evidence, nevertheless, is of sufficient substance to justify a future comprehensive investigation of these suspected materials which are rather widely distributed in the human environment[3,12] for their possible carcinogenic effects on the bladder of man and animals.

Conclusions

1. Precancerous lesions and benign and malignant neoplasms of the skin, connective tissue, stomach, and bladder developing in mice, rats, and guinea pigs, respectively, after their exposure to soot from coffee-roasting plants, or its residue of acetone extract by the cutaneous, intramuscular, and respiratory routes, attest to the carcinogenic property of this air pollutant.

2. The occurrence of cystitis cystica, papillomas, and carcinomas in the bladders of guinea pigs inhaling and ingesting finely powdered soot from coffee-roasting plants make this material suspect of containing a carcinogen or carcinogens excreted in the urine.

3. From a critical analysis of experimental and epidemiologic data, it is not likely that effluents from coffee-roasting plants in New Orleans are a major causal factor of the unusually high mortality and morbidity rates of cancers of the lung, larynx, and bladder observed among the resident population of this city. It is, on the other hand, possible that this air pollutant assumes a contributory role in this respect.

Environmental Cancer Section, National Cancer Institute.

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"Extramammary Paget's Disease" of the Anorectal Junction

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"Extramammary Paget's disease" of the anus occurs with great rarity. There is, as yet, no general consensus as to the pathogenesis of this condition in spite of a more generally accepted concept relating to the genesis of mammary Paget's disease. The present case is of particular interest because of our inability to demonstrate a relationship between the intraepithelial neoplasm and the underlying eccrine or apocrine glands.

Report of a Case

A 62-year-old white woman was admitted to Baylor University Medical Center on Aug. 9, 1959, for a hemorrhoidectomy and wide excision of the perianal skin, which seemed to be involved in a typical chronic pruritus ani. About eight years before, mild pruritus ani developed, which had become severer for the last three years. Small amounts of bood had been noticed on the stool and toilet paper from excoriation of the pruritic skin for the past two years. There had been protrusion of anal masses at the stool, with spontaneous reduction each time, for the last two years. The patient's general health was excellent, and there were no other complaints. An anal fissure and/or ulcer had been excised under local anesthesia in 1942 by her family physician; however, a pathologic examination was not performed.

Preoperative examination revealed large internal hemorrhoids and smaller external hemorrhoids. The anorectal skin was thickened, grayish, edematous, and excoriated, presenting a classical picture of chronic pruritus ani. The process extended externally for approximately 6.5 cm. from the anal mucocutaneous junction. The rectal mucosa above the hemorrhoids was completely normal for 24 cm., as visualized by endoscopic examination.

Excision of the hemorrhoidal masses, together with large wedges of perianal skin, was performed. After pathologic examination of the excised tissue revealed "extramammary Paget's disease of the anus," all the skin of the anus was excised from the normal mucosa externally for a distance of 1 cm. into normal tissue beyond the grossly thickened, pruritic skin. The patient is to be followed closely, and repeated biopsies will be done as indicated. If the process recurs, an abdominoperineal resection of the rectum and anus will be done. There has been no evidence of recurrence during the seven months since surgery.

Pathologic Findings

The tissues removed at the first operation were serially divided into strips 3 mm. thick, and subserial microscopic sections were made through the paraffin blocks of the entire specimen. Forty-one radial sections were made through the perianal tissues removed at the second operation, and subserial microscopic sections were prepared from all paraffin blocks.

The neoplastic process began in the transitional and squamous epithelium just distal to the normal rectal mucosa and extended to within 1 cm. of the distal margin of anal tissue excised at the second operation. The entire circumference of the perianal epithelium was involved in the process with strips of normal epithelium interposed between areas infiltrated by tumor cells. In most areas the epithelium involved by tumor was thickened and covered by a dense layer of keratin (Fig. 1); in other places the epithelium was of normal thickness or thinner than normal. No areas of ulceration were seen. Parakeratosis and acanthosis were prominent features, and the

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Fig. 1.—Perianal skin showing hyperkeratosis, Pagetization of epithelium, elongation and broadening of rete pegs, and chronic inflammation of dermis. Hematoxylin and eosin; reduced to 63% of mag. × 35.

Fig. 2.—Parakeratosis and hyperkeratosis with pagetization of epithelium. Hematoxylin and eosin; reduced to 63% of mag. × 100.

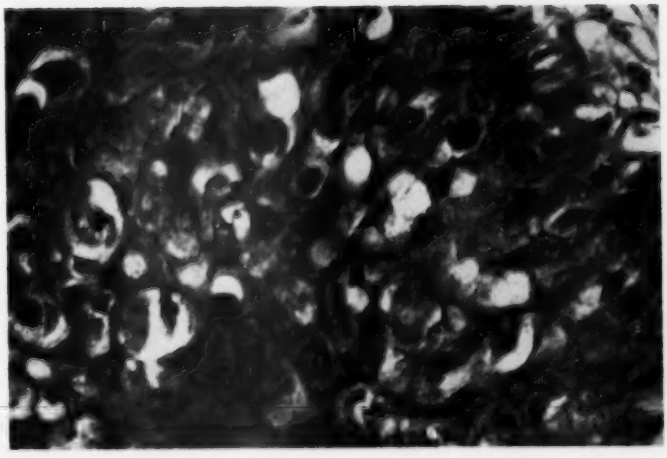

Fig. 3.—Pagetoid cells with pleomorphic vesicular or hyperchromatic nuclei and vacuolated or clear cytoplasms Hematoxylin and eosin; reduced to 63% of mag. × 400.

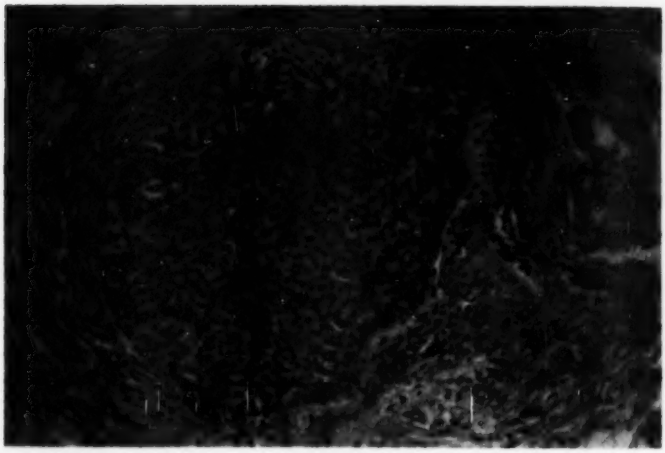

Fig. 4.—Pagetoid cells in duct of sweat gland. Hematoxylin and eosin; reduced to 63% of mag. × 100.

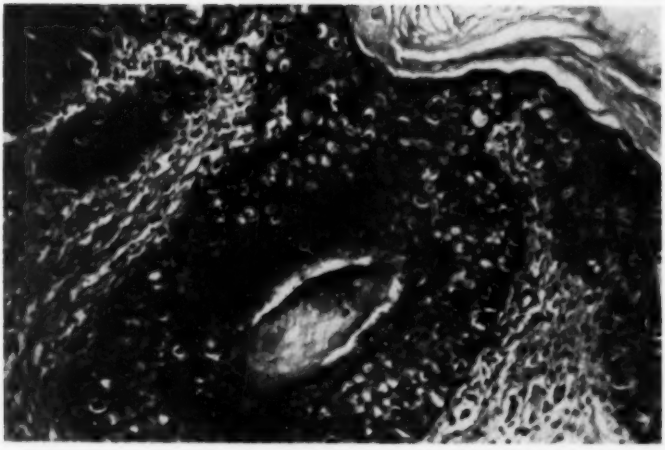

Fig. 5. — Presence of Pagetoid cells in hair follicle. Hematoxylin and eosin; reduced to 63% of mag. × 100.

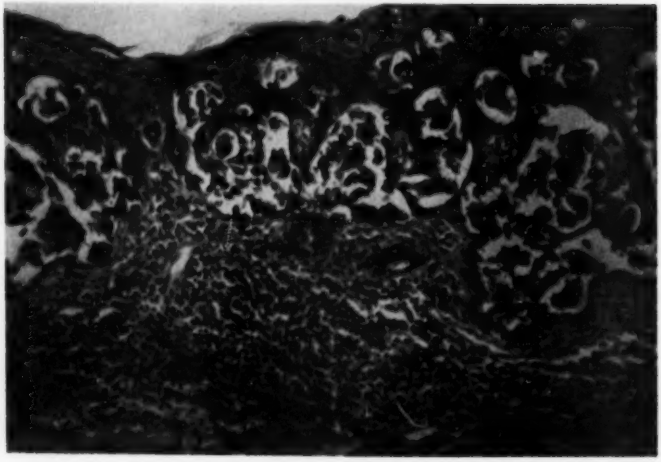

Fig. 6. — Intraepithelial glandular spaces lined at base by columnar glandular cells and containing papillary cellular processes. Hematoxylin and eosin; reduced to 63% of mag. × 100

rete pegs were conspicuously broadened (Fig. 2). The dermal papillae were edematous, infiltrated by moderate to marked numbers of lymphocytes, and contained myriads of thin-walled capillaries distended with blood.

Tumor cells were present in all layers of the epithelium but displayed a predilection for a parabasal location. The cells were distributed singly and in compact clusters, occasionally causing complete displacement of the squamous cells throughout the entire thickness of the epithelial layer. The cells were larger than adjacent squamous cells and displayed a vacuolated or clear cytoplasm with indefinite cell membranes (Fig. 3). The oval or rounded nuclei were usually vesicular, occasionally hyperchromatic, moderately pleomorphic, and contained one or more small, dense nucleoli. The cells were devoid of pigment and did not form keratin or intercellular bridges. Mitotic forms were present in moderate numbers. No invasion of the subepithelial tissues was encountered; however, tumor cells extended downward in the squamous lining of ducts of eccrine and apocrine glands (Fig. 4) and into the squamous lining of hair follicles (Fig. 5). Of great interest was the formation of glandular spaces, lined at the base by low columnar cells (Fig. 6). A meticulous search failed to reveal atypical cells in the eccrine or apocrine glands. Periodic acid-Schiff (PAS) stains before and after diastase digestion revealed strongly positive material in the cytoplasm of many of the cells.

Comment

The detailed histologic studies of Grinvalsky and Helwig revealed the presence of PAS-positive and mucicarminophilic columnar and goblet cells in the anorectal junction.[1] Rabson, Van Scott, and Smith[2] subsequently recorded a case of "extramammary Paget's disease" of the anorectal junction which originated from the mucus-secreting cells of the surface epithelium, anal ducts, and glands and an apocrine sweat gland. As mucus-producing cells are normally present in the surface epithelium, it is reasonable to assume that an intraepithelial adenocarcinoma could arise from these cells without concomitant origin in the apocrine or eccrine glands. The present case, we believe, represents an example of such a case. The downward migration of tumor cells into the ducts of the subepithelial eccrine and apocrine glands is histologically, and possibly histogenetically, similar to the involvement of mucus glands and their ducts in intraepithelial carcinoma of the uterine cervix. Surface epithelial involvement in some cases of anorectal Paget's disease possibly reflects an upward migration of tumor cells originating in the sweat glands. However, we are of the opinion that surface epithelial involvement in many cases represents neoplasia of preexisting glandular cells developing concomitantly with a similar process in the sweat-gland epithelium.

Ten previously recorded cases of "extramammary Paget's disease" of the anorectal junction were found in a review of the literature.[2-11] The commonest symptom was chronic pruritus; the clinical appearance of the involved skin was consistent with the excoriating, lichenifying, erythematous picture of chronic pruritus ani. Several patients exhibited external and internal hemorrhoids, and a history of rectal bleeding was frequently obtained. The disease is apparently commoner in males, and most of the patients were in their sixth decade of life when the diagnosis was established. Regional and widespread metastases have been observed.

As the clinical management of "extramammary Paget's disease" of the anus differs from Bowen's disease and melanoma, it is essential that a histologic differentiation of these three conditions be made. In Bowen's disease the cells do not contain PAS-positive material; they do produce keratin and join adjacent squamous cells by intercellular bridges. The stroma may become invaded in Bowen's disease, but this has not been reported in adenocarcinoma of the extramammary Paget's type. The lesion in "extramammary Paget's disease" is usually diffuse in the perianal region, in contrast to the localized distribution of

melanomas, and, according to Lund, the demonstration of mucin within the Pagetoid cells indicates that the lesion is not a melanoma.[12]

The demonstration of a malignancy in this case was unsuspected clinically, and the importance of routine histologic examination of all tissues removed at surgery is again emphasized.

Summary

A case of "extramammary Paget's disease" of the anorectal junction without involvement of the underlying eccrine or apocrine glands is presented. We believe that the carcinoma began in glandular cells that are normally present in the surface epithelium of the anorectal junction.

The histologic and cytochemical procedures were performed by Freida Carson, H.T. (ASCP).

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Experimental Renal Papillary Necrosis

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The condition known variously as renal papillary necrosis, necrotizing papillitis, and medullary necrosis of the kidney was first described by von Friedreich [1] in 1877. In 1937 Günther [2] drew attention to the association of the disease with diabetes mellitus, and since then this relationship has been well recognized; Robbins and Angrist [3] estimate that papillary necrosis is found in 3.2% to 5% of diabetics coming to necropsy. In nondiabetics the incidence is lower; the condition is then usually associated with urinary obstruction. In the great majority of cases, diabetic and nondiabetic, the lesion accompanies acute suppurative pyelonephritis; Robbins and Angrist [3] estimate that it complicates 4% of fatal cases. Papillary necrosis has, however, been found in the absence of both diabetes and urinary obstruction,[4-11] and occasional cases occur in the absence of pyelonephritis.[11-13]

Among the striking features of renal necrosis of this type are the close resemblance of the lesions to infarcts and their localization to the medulla; indeed, they are often limited to the papillae and may involve only portions of these structures. These characteristics have caused many previous authors [7,13-17] to stress the vulnerability of the papillary blood supply; small, thin-walled vessels carry arterial blood along the papillae and pass directly into returning veins by U-shaped loops. Compression of these vessels by interstitial edema and inflammatory infiltrate or by raised intrapelvic pressure has been suggested as the cause of ischemic papillary infarction. Arteriosclerotic narrowing of renal vessels and diabetic glomerulosclerosis have also been postulated as causes of papillary ischemia.[7,17,18] Venous and capillary thrombi have sometimes been observed in association with papillary necrosis.*

In the course of another series of experiments in rats, in which ligation of renal veins and of renal arteries and their main branches was performed, it was observed that there were clear-cut differences between the distribution of infarcts which resulted from occlusion of veins and the distribution of infarcts which resulted from occlusion of arteries; arterial infarcts always involved the whole thickness of the cortex, as well as the medulla, whereas venous infarcts involved the medulla, with only limited spread to the adjacent cortex. The close resemblance of the lesions in the latter instance to renal papillary necrosis in man prompted an investigation of the effects upon the rat's kidney of partial and complete obstruction of the renal vein.

Experimental Procedures

Male rats of the Sprague-Dawley strain, weighing 265 to 400 gm., were employed. Operations were performed under ether anesthesia. A full aspetic technique was not carried out, but instruments were autoclaved before each operating session. The fur was clipped from a large area of skin, the center of which was the left flank. Healing of the surgical incisions always proceeded satisfactorily, and no evidence of infection was found externally or internally in any rat at autopsy.

In one series of experiments (Group A, Table 1) the left renal veins were occluded completely by ligatures. The rats were killed at intervals of two to six days, with the exception of one rat, which

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* References 7, 11, 12, 15, 17, 19, 20.

TABLE 1.—*Group A: Renal Veins Completely Occluded by Ligature*

Duration of Experiment	No. of Animal	Results
6 hr.	1	Intense hyperemia of boundary zone; early necrotic changes in medullary tubular epithelium
2 days	2- 6	Infarction of whole medulla, occasionally sparing tubules at the tip; occasional minor cortical extensions; congestion and hemorrhage in boundary zone
4 days	7- 9	Infarction of whole medulla with minor cortical extensions; early fibroblastic proliferation in boundary zone; little remaining congestion and hemorrhage
5 days	10-11	Little congestion and hemorrhage in boundary zone; well-marked fibroblastic proliferation
6 days	12	Marked fibroblastic proliferation in medulla

died spontaneously some six hours after operation. The blocks of tissue selected for section were central cross sections of the left kidneys, cut so as to include papillae; they were fixed in Bouin's solution. Serial sectioning of the kidneys was not performed because it proved to be easy to see with the naked eye the distribution of infarcts in thin, parallel slices cut across each kidney.

In another series of experiments (Group B, Table 2) the left renal veins were partially occluded; in each case a ligature was tied firmly around both the vein and a piece of wire of 1 mm. diameter. The wire was withdrawn immediately after tying the ligature. The rats were killed at intervals of two to nine days after operation. Blocks of tissue were selected and fixed by the same methods as were used for the kidneys in Group A.

Results

The results of individual experiments are outlined in the Tables.

The left kidneys of 12 rats (Group A) in which the renal veins were completely occluded all showed infarction. In six rats there was total infarction of the medulla with some extension of necrosis into the cortex; the areas of cortical spread were circumscribed, were usually placed a little to one side of the center of the kidney, and seldom reached the capsule. In one rat, a small portion of the medulla on one side remained viable, while the remainder was

TABLE 2.—*Group B: Renal Veins Partially Occluded by Ligature*

Duration of Experiment, Days	No. of Animal	Results
2	1- 8	Infarction of whole or part of medulla with scattered extensions into cortex; occasional surviving tubules at tip of papilla
2	9, 10	No massive infarction but degenerative and regenerative changes in tubules; interstitial edema and leukocytic infiltration; protein casts in tubules
3	11	Infarction of whole medulla
4	12, 14	Infarction of whole medulla; congestion and hemorrhage boundary zone; early fibroblastic proliferation
4	13	No massive infarct; degenerative and regenerative changes in medullary tubules; slight interstitial edema and protein casts in tubules
6	15-16	No massive infarction; regenerative changes in tubules; edema
7	17	Small infarct at center of pyramid; fibroblastic proliferation at margin of infarct
8	18-19	Infarction of papilla with some regenerative activity and fibroblastic proliferation
9	20	Infarction of whole medulla
9	21-22	Infarction of whole papilla with scattered extension into rest of medulla and occasional surviving cells in papilla
9	23	No massive infarction; mild regenerative activity in papilla with some edema and leukocytic infiltrate

Figs. 1-6.—Midtransverse sections of kidneys. Hematoxylin and eosin; ×6.

Fig. 1.—Group B, Rat 20. Nine days after partial occlusion. Total infarction of medulla.

Fig. 2.—Group B, Rat 17. Seven days after partial occlusion. Small infarct occupies central area of pyramid.

necrotic and infarction extended to a limited area of the cortex. In five rats there was total infarction of the medulla with no cortical extension; portions of a few tubules occasionally survived at the extreme tips of the papillae. It will be apparent that the extent of infarction produced by complete occlusion of renal veins was somewhat

Fig. 3.—Group B, Rat 19. Eight days after partial occlusion. Infarction of the papilla except for a few tubules at the extreme tip. Slight extension upward of infarction from papilla into remains of pyramid.

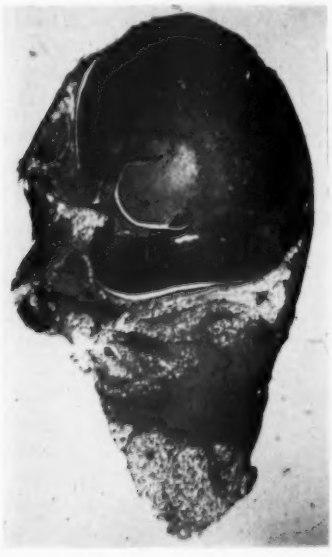

Fig. 4.—Group A, Rat 11. Five days after complete occlusion. Total infarction of medulla.

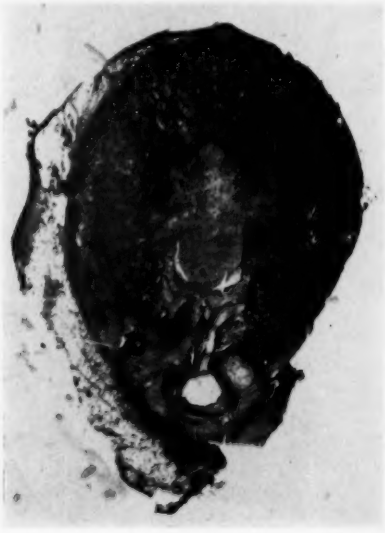

Fig. 5.—Group B, Rat 12. Four days after partial occlusion. Infarction of medulla with small cortical extension on one side.

Fig. 6.—Group B, Rat 21. Nine days after partial occlusion. Infarction of whole papilla except a few tubules at extreme tip. Slight upward extension of infarction from papilla into central part of remainder of pyramid.

variable, but it always centered upon the medulla; the variation is probably related to differences in the extent of renal vein anastomoses in individual rats.

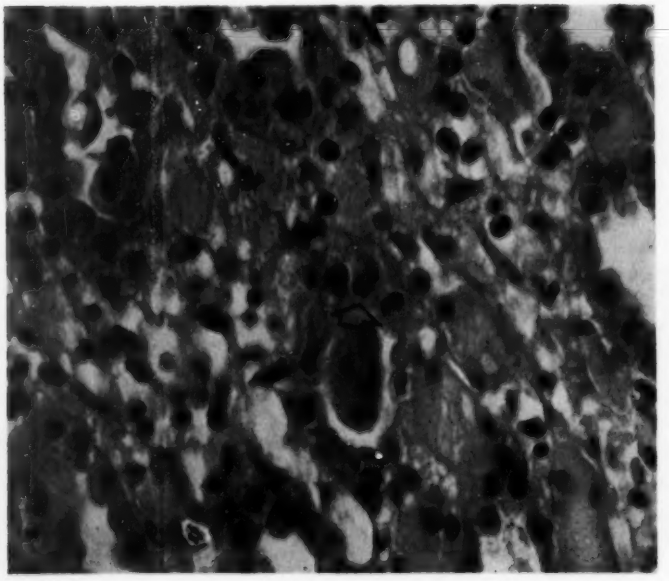

Fig. 7.—Hematoxylin and eosin. × 400. Mitotic and regenerative activity in papillary tubules.

Twenty-three rats were subjected to partial occlusion of left renal veins. At autopsy in all cases the veins were found not to be thrombosed; blood could be made to pass back and forth past the sites of the ligatures by gentle pressure. No venous thrombi were observed on microscopy of the kidneys; veins seen in section were markedly dilated and engorged, the arcuate veins being especially prominent.

The left kidneys of 17 of the 23 rats in Group B showed infarction. Four kidneys showed total medullary infarction with limited cortical spread. Six kidneys showed total infarction of the medulla only. Seven kidneys showed infarcts confined to limited areas of the pyramids; these might involve the whole of a papilla or might occupy the central area of a pyramid, being placed mostly within a papilla. Infarcts of this kind closely resembled those seen in papillary necrosis in man. In rats killed within four days after operation there was intense vascular congestion and much hemorrhage at the margins of the renal infarcts, especially when they coincided with corticomedullary boundaries. Considerable marginal leukocytic infiltration was also present. Thereafter, the vascular changes became progressively less, and marginal fibroblastic and endothelial-cell proliferation appeared and increased. In kidneys containing large medullary infarcts, cortical tubules early showed focal degeneration changes, ranging from cloudy swelling to necrosis; in more prolonged experiments focal atrophy of nephrons was seen in the cortices. These findings are similar to those of Mandel and Popper [21] in the kidneys of rabbits injected with vinylamine.

In the remaining 6 of the 23 rats in Group B, with partially occluded left renal veins, no circumscribed infarcts developed; but there was congestion of the boundary zones, and the papillae showed edema and light leukocytic infiltration of the interstitial tissue, dense protein casts within tubules, and degeneration, necrosis, and regeneration of tubular epithelium, with focal tubular disruption. These changes were ascribed to anoxia, sufficient to cause tissue damage in the papillae, but inadequate to produce massive infarction.

In this series of experiments, also, there was a variation in the extent of the necrosis produced. Here, again, it may be ascribed partly to individual variation in venous anastomotic communication, but there are other factors which must be taken into account. There were differences in size of the left kidneys and the associated renal veins, and consequently there must have been variations in size of the residual lumina of the constricted veins. The ligatures also were tied by hand, and therefore some variation in the tightness of the ties must have been present.

No kidney in either Group A or Group B showed any macroscopic or microscopic evidence of suppuration, and no organisms were seen in Gram-stained sections.

A number of kidneys in both Group A and Group B showed narrow, band-like areas of cortical necrosis immediately under parts of the capsules; these were always beneath capsular hematomas and were believed to be the result of superficial trauma produced by the handling of the kidneys at operation.

Comment

A number of attempts have been made to reproduce experimentally the lesions of renal papillary necrosis.

Muirhead et al.[16] produced hydronephrosis and papillary necrosis in 18 of 28 dogs in which ureteric ligation was performed. Perfusion with India ink demonstrated poor filling of medullary blood vessels in the presence of raised intrapelvic pressure. Edmondson and associates [15] however, were unable to produce the lesion by ureteral ligation in rats, even after pancreatectomy.

Robbins and Angrist,[3] believing that the disordered fat metabolism of diabetes might be a factor in the pathogenesis of papillary necrosis, cited the experiments of Burr and Burr [22] with rats fed on virtually fat-free diets; among other disorders, these rats de-

veloped hematuria. Borland and Jackson [23] described the renal changes in such rats; they included necrosis of papillary tips accompanied by calcification, but lesions in the cortical tubules were also present. Herbut et al.[24] have recorded kidney lesions in alloxan diabetes; tubular necrosis occurred but was confined to the cortex, being absent in the medulla.

Rehns [25] produced papillary lesions in rabbits and guinea pigs by injections of tetrahydroquinoline; necrosis was, however, usually localized to the peripheral portions of the papillae and did not affect the central areas.

Levaditi,[26] using rabbits, goats, guinea pigs, mice, and rats as experimental animals, induced renal medullary necrosis by injection of vinylamine. More recently, Mandel and Popper [21] have administered vinylamine to 36 rabbits and 1 dog; medullary necrosis was produced in the majority of the animals and usually involved the whole of the medulla. These authors record that in the early stages of poisoning there was intense medullary congestion and cortical ischemia with focal changes in cortical tubules, ranging from cloudy swelling to necrosis. The blood content of the cortical vessels later returned to normal, while necrosis of the medulla appeared and extended to the boundary zone. These authors remark that "the mechanism by which extreme hyperemia in the medulla changes into necrosis is not . . . so obvious. One feels compelled to invoke as connecting links prolonged vasoparalysis, formation of conglutination thrombi, . . . and tissue anoxia." Our results bear a considerable resemblance to those of Mandel and Popper,[21] both in the production of necrosis in the absence of any preceding or complicating infection and in the predominant localization of necrosis to medullary tissue. However, in some of our rats in which renal veins were partially occluded, infarcts were small, and involved only portions of the pyramids, and these lesions perhaps resembled more closely renal papillary necrosis in man. Mandel and Popper [21] concluded that in their experiments some vascular disturbance, consequent upon vinylamine poisoning, must have been the cause of the medullary necrosis, and it appears from our results that obstruction of the venous drainage of the kidney is able to produce diffuse or localized infarction of the medulla of the rat's kidney. The vascular arrangement of the rat's kidney appears to be essentially similar to that obtaining in man (Trueta et al.[27]), and it therefore seems probable that some form of venous obstruction may be the cause of the infarct-like lesions of human papillary necrosis.

Of 12 consecutive cases of papillary necrosis, collected from the autopsy files of the Salt Lake General Hospital, we have observed organizing thrombi in small veins in the kidneys of 3 cases and tubulovenous anastomoses in 2 cases. All available blocks of renal tissue were examined, but this study was a retrospective one, and it is therefore possible that more extensive sectioning of the affected kidneys might have revealed additional thrombi. A number of previous authors have observed such thrombi,* but there are many cases of papillary necrosis on record in which thrombi have not been seen. There are, however, occasional cases in the literature [11,12] in which bilateral widespread papillary necrosis has been found in the absence of diabetes, urinary obstruction, or renal infection, but associated with extensive thrombosis of the larger tributaries of the renal veins. These cases are of particular interest in connection with our experimental findings in rats, because they indicate that venous obstruction alone may cause papillary necrosis in man.

It has frequently been suggested that interstitial edema and inflammatory-cell infiltration, with or without raised pressure in the renal pelves, might cause medullary ischemia by compressing the small vessels of the renal medulla and obstructing the arterial blood supply [7,14-17]; it seems more

References 7, 11, 12, 15, 17, 19 and 20.

probable that small veins might be occluded partially or completely by such a mechanism.

Heppleston [13] has reported a case of renal papillary necrosis which complicated necrotizing angiitis and which occurred in the absence of diabetes, urinary obstruction, and renal infection. Acute focal tubular necrosis was also present and was believed to have antedated the papillary necrosis, and it is suggested that the tubular and interstitial changes associated with acute tubular necrosis caused sufficient reduction in blood flow to the papillae to induce infarction therein. Heppleston [13] believed that in cases of suppurative pyelonephritis, vomiting and dehydration, consequent upon uremia, may cause acute tubular necrosis and that this, in turn, may cause sufficient added vascular obstruction to produce infarction of the renal papillae. In seven of our human cases of papillary necrosis there is evidence of tubular necrosis and regeneration in areas of the renal boundary zones, and these tubular lesions are associated with interstitial edema and intense congestion of small vessels and retention of leukocytes within them. We agree with Heppleston [13] in believing that this provides a possible mode of vascular obstruction, but in view of our experimental findings we believe that blockage of venules is probably of more importance than arterial occlusion.

Summary

Partial and complete occlusion of renal veins have been produced in rats by ligation.

Complete occlusion produced total infarction of the medulla with some cortical extension of infarction in a proportion of cases.

Partial occlusion produced a range of changes.

1. Total infarction of the medulla with slight cortical extension
2. Total infarction of the medulla confined to the medulla
3. Focal infarcts in the pyramids
4. Focal necrosis of tubular epithelial cells in the papillae

It is suggested that renal papillary necrosis in man is the result of venous obstruction.

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Arterial Changes in Pulmonary Sarcoidosis

L. MICHAELS, M.D., B.S., D.Path.; N. J. BROWN, M.B., M.R.C.P., and M. CORY-WRIGHT, M.B., B.S., London, England

In recent years a considerable amount of clinical and laboratory investigation has been carried out on Boeck's sarcoidosis. The clinical and histological criteria of diagnosis have been sharpened, and there have been biochemical and therapeutic advances.[1] Yet, while the disease seems to form a definite clinical entity, the difficulty still remains that the tuberculoid histology on which the diagnosis of sarcoidosis depends is a manifestation of many etiological agents. The observation of any new histological pattern in sarcoidosis is of interest, since it may help in the classification of sarcoid conditions or throw some light on their pathogenesis. For this reason we report a granulomatous change in branches of the pulmonary artery that we have observed in two cases of pulmonary sarcoidosis.

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Report of Cases

CASE 1.—*History*

A woman aged 36 had been coughing up increasing quantities of sputum since an attack of pneumonia eight months previously. She had suffered from a frequent cough since she had left-sided pleurisy 14 years before. She was unduly short of breath on exertion and had been feeling tired in recent months. On examination the only abnormal physical signs were scattered crepitations over the left axilla and left lung base. A bronchogram showed marked saccular bronchiectasis in the left lower lobe and both divisions of the lingula. A left lower lobectomy and removal of the lingula were performed; in the course of the operation hilar lymph nodes were removed to obtain better exposure. Postoperative progress was uneventful, and five months after the operation she was in good general health, with no cough or sputum.

Description of Specimen

The specimen consisted of the left lower lobe, 8×14×2.5 cm.; the lingula, 3×8×1 cm., and

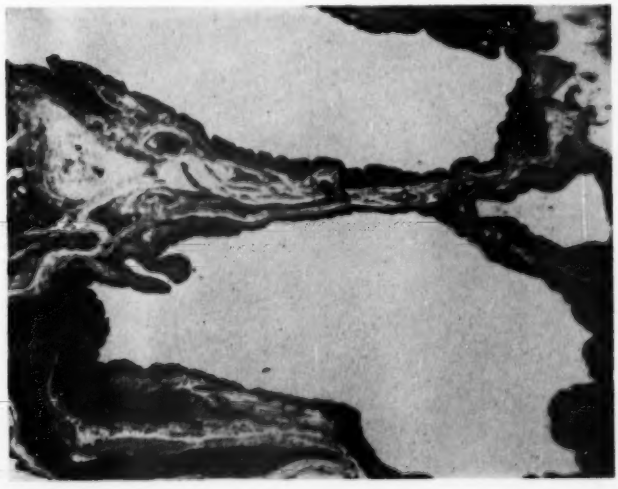

Fig. 1 (Case 1).—Bronchiectatic cavities. Hematoxylin-eosin stain; ×80.

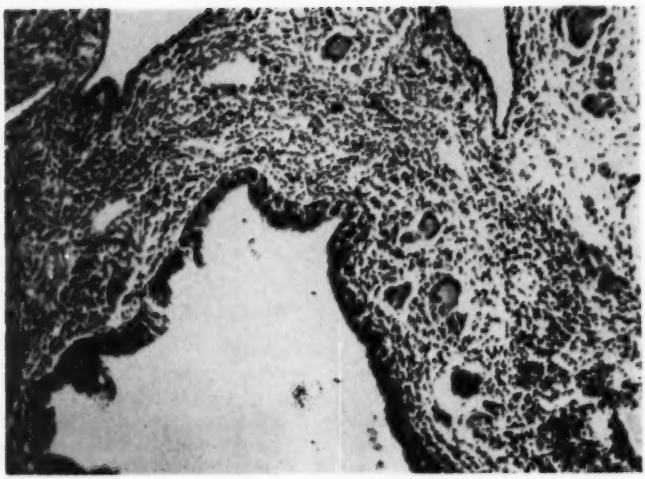

Fig. 2 (Case 1).—The bronchiectatic spaces are lined by bronchial epithelium, beneath which there are tuberculoid foci. H. & E.; ×240.

seven hilar lymph nodes. There were several subpleural emphysematous bullae. The cut surface of the lung showed marked bronchiectasis in all areas. Between the bronchiectatic cavities the lung tissue was rather pale. The lymph nodes were all moderately enlarged and firm; they showed gray cut surfaces and only small quantities of carbon pigment.

Histological Appearances

The lung shows bronchiectasis (Fig. 1) and also a chronic granulomatous lesion, suggestive of sarcoidosis.

The bronchiectatic cavities are lined by tall columnar epithelium, beneath which there is infiltration with lymphocytes, plasma cells, and occasional foci of epithelioid cells and Langhans and foreign-body giant cells (Fig. 2). In some places there are lymphoid follicles with germinal centers near the epithelium of the bronchial walls.

Many alveolar septa, especially around larger bronchi, are greatly thickened by focal collections of epithelioid cells and Langhans giant cells surrounded by and infiltrated with lymphocytes, plasma cells, eosinophils, and polymorphonuclear cells. In some areas this process has produced a fibrous thickening of alveolar septa. There

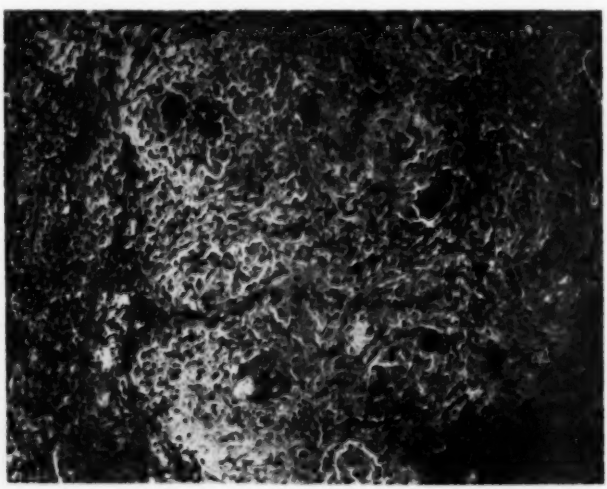

Fig. 3 (Case 1).—Hilar lymph node containing many tuberculoid follicles. H. & E.; ×240.

is no caseation. Foam cells are present in some alveoli, and there is a moderate degree of emphysema. Neither stellate formations nor Schaumann bodies are seen within the giant cells. Examination of lung sections between crossed polaroids shows no birefringent mineral material. No tubercle bacilli are seen in Ziehl-Neelsen-stained sections. Fungi are not detected after staining sections with periodic acid-Schiff (PAS) technique.

The lymph nodes are extensively infiltrated with tuberculoid nodules, consisting of epithelioid cells and Langhans and foreign-body giant cells with scattered lymphocytes; there are large areas of fibrosis (Fig. 3).

There are many focal lesions of pulmonary arteries. The changes are most conspicuous in the media of small arteries. In some of the lesions the most striking change is a separation of the smooth muscle cells by a highly vacuolated, noncellular basophilic material resembling mucin, which is metachromatic with toluidine blue. The vacuoles, which may be as large as 20μ in diameter, are usually empty but in some places contain a granular basophilic material. There is a moderate infiltration in these and other areas with epithelioid cells, plasma cells, and lymphocytes. In some of the lesions the infiltrate is more intense, and among the inflammatory cells are found foreign-body giant cells. At these points also there is seen a minor degree of thickening of the intima by fibroblasts. Elastic stains usually show rupture of the internal elastic lamina in these situations; elastic fibers are not seen in close relation to giant cells, as has been described in cases of giant-cell arteritis, and rupture of the internal elastic lamina is sometimes seen in the absence of giant cells in the adjoining infiltrate (Figs. 4 and 5). In no section is there seen any serious obstruction of the vascular lumen by the granulomatous process in this case. An occasional small vessel is present which shows severe intimal thickening, but the internal elastic lamina is intact and the media shows no inflammatory change. It is considered that such intimal fibrosis is a nonspecific effect of the inflammatory change, and is not a sarcoid lesion. The adventitia shows no abnormality. The vascular foci can nowhere be seen to be continuous with similar lesions in the surrounding lung tissue.

CASE 2.—*History*

A man of 37 was admitted to the hospital for abdominal pain, which had been present for 24 hours before admission. An enlarged spleen was detected with barium meal examination. A laparotomy was refused at this admission, but two months later the patient was readmitted complaining of abdominal pain of sudden onset, and laparotomy was performed, revealing a perforated anterior peptic ulcer, which was closed. The patient com-

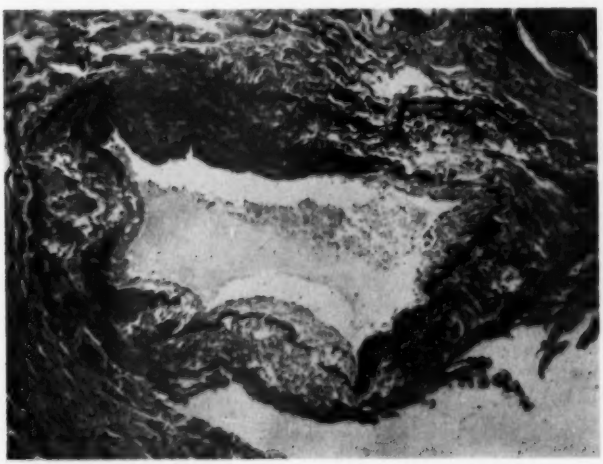

Fig. 4 (Case 1).—A pulmonary artery showing granulomatous lesions in media, with rupture of internal elastic lamina and intimal fibrosis. Elastic tissue stain; × 240.

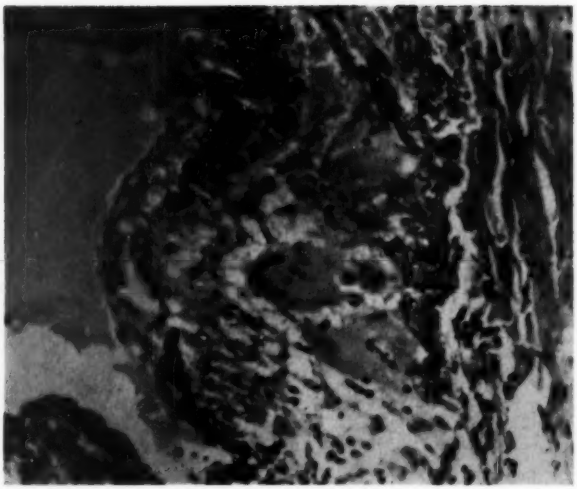

Fig. 5 (Case 1).—Giant and epithelioid cell infiltration of media of pulmonary artery with rupture of adjacent internal elastic lamina and thickening of intima. Elastic tissue stain; ×830.

plained of a cough which had troubled him for two years, and x-ray of the chest showed generalized lung lesions, suggestive of fibrosis. Two months after operation his liver and spleen were found to be enlarged. Liver biopsy revealed the changes of sarcoidosis; he was therefore treated with streptomycin, aminosalicylic acid, and cortisone. Ten months later he had an epileptic attack while under reinvestigation in hospital; he was therefore treated with sodium diphenylhydantoinate (Epanutin). His liver and spleen were still enlarged, but the chest x-ray showed less mottling of lung fields. Nine months later, that is, two years after his first admission, he developed numerous epileptic attacks during the night and died on arrival at hospital.

Autopsy Findings

There were fibrous adhesions between the first part of the duodenum and the inferior surface of the liver. The lungs were rather pale; they were granular on palpation and showed flat subpleural nodules up to 5 mm. in diameter (Fig. 6), but on the cut surfaces no actual lesions could be seen with the naked eye. The hilar lymph nodes were slightly enlarged. The spleen was greatly enlarged (910 gm.). It showed a nodular surface, and on the cut surface were numerous pale pink nodules 1 to 2 cm. in diameter and firm in texture. There were numerous enlarged lymph nodes in the neck, in the mesentery, along both sides of the abdominal aorta, and around the stomach. The lymph nodes

Fig. 6 (Case 2).—Pleural surface of right lung showing flat nodules up to 5 cm. in diameter. Reduced to 57% of mag. ×3.

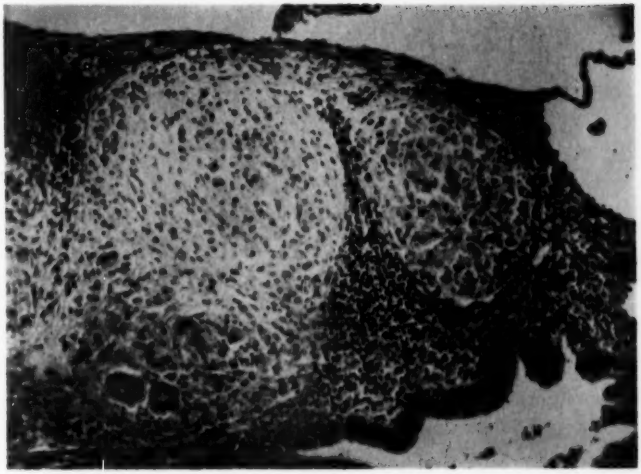

Fig. 7 (Case 2).—Infiltration of bronchiolar wall by sarcoid tubercles. H. & E.; × 240.

were up to 2 cm. in diameter, were usually discrete, but were occasionally matted together, and showed a uniformly pink cut surface. The liver was moderately enlarged (2,320 gm.), and in its cut surface it showed numerous pale red, fine nodules 2-5 mm. in diameter. There was the scar of a healed ulcer in the first part of the duodenum, and the surrounding tissues were densely adherent at this point. The brain showed no abnormality apart from congestion of vessels.

Histological Findings

Scattered throughout the lung tissue are numerous groups of follicular lesions composed of Langhans giant cells and epithelioid cells; each is surrounded by and slightly infiltrated with lymphocytes and plasma cells, and there is a considerable amount of collagen deposition around the periphery. The lesions are seen in alveolar walls and under the epithelium of bronchi and bronchioles. There is no caseation. Very occasional asteroid bodies are present in giant cells; Schaumann bodies are absent. No tubercle bacilli are seen in Ziehl-Neelsen-stained sections. The lymph nodes are infiltrated with numerous lesions of similar type, and there are large masses of hyaline collagen (Fig. 7).

Similar lesions are present in the walls of moderate-sized branches of pulmonary arteries. They are, as in Case 1, of an appearance similar to that of the lesions

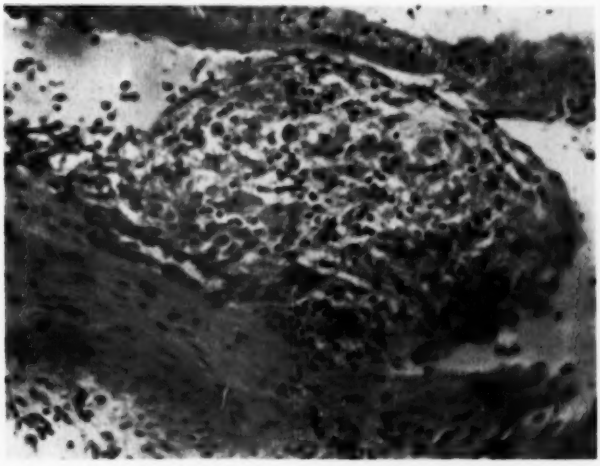

Fig. 8 (Case 2).—A granulomatous focus projecting into lumen of a pulmonary artery. H. & E.; × 600.

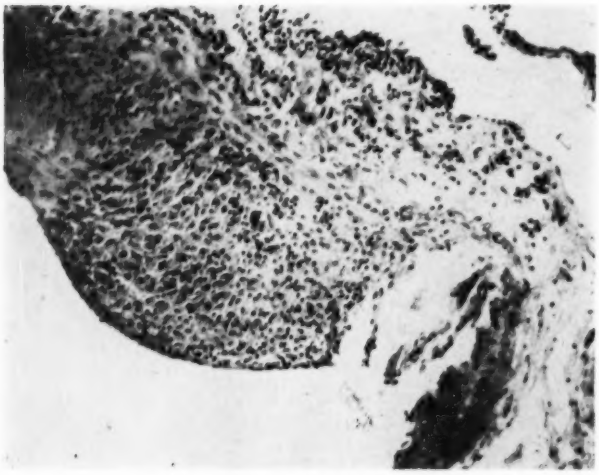

Fig. 9 (Case 2).—Sarcoid focus in media of a pulmonary artery. H. & E.; × 240.

elsewhere in the lung, consisting of Langhans and foreign-body giant cells, epithelioid cells, and lymphocytes, and are situated either in the media or in the intima. They are of larger size than the vascular lesions in Case 1, and some of them project into the lumen of the vessel (Figs. 8 and 9). Fibrous thickening of the intima is present over the lesions in some places, and occasionally there is thrombosis of blood in the lumen. The internal elastic lamina shows complete disruption in the region of the granulomatous foci. In some areas in this case the vascular inflammatory process is seen to be directly continuous with lesions in the surrounding lung. In other areas vessels showing lesions are adjacent to normal lung tissue, as in Case 1.

The splenic and hepatic nodules consist of giant-cell granulomatous foci similar in appearance to the lung lesions. A few such foci were also found in the kidneys. Arterial lesions were not found in any organ except the lungs.

Comment

The clinical and pathological features of these two cases are typical of Boeck's sarcoidosis. In neither case was the disease process responsible for fatal or crippling injury. In Case 1 the lesions producing symptoms and necessitating lobectomy were those of bronchiectasis; sarcoidosis was diagnosed on subsequent histological examination of the specimen. In Case 2, although there was widespread involvement by the granuloma, the lesions seemed to be slowly resolving on treatment, and death was caused by an attack of status epilepticus, apparently unrelated to his sarcoidosis. In both cases the distribution and appearances of the tuberculoid lesions were typical of sarcoidosis except for the arterial involvement. The heavy involvement of the hilar lymph nodes, the discrete character of the nodules there, the absence of tubercle bacilli or any other organisms, and the lack of caseation in the pulmonary lesions of the type described, are characteristic. The appearances of the hepatic and splenic lesions in Case 2 are similarly typical of sarcoidosis.

The unusual feature of the cases here described is the patchy involvement of branches of the pulmonary artery by the giant-cell granulomatous process. The inflammatory infiltrate is found in the media or intima. Later there are destruction of the internal elastic lamina and fibrosis of the intima. Thrombosis of blood in the lumen may occur.

This appearance would seem to be rare, since we have found the report of only a

single comparable case in the literature.[2] In this case a submandibular swelling in a man of 32 showed a histological appearance typical of sarcoidosis. In addition, a number of muscular arteries included in the inflammatory tissue were affected by a medial granulomatous infiltration of a type similar to the surrounding tuberculoid change. There were rupture of the internal elastic lamina and intimal fibrosis of sufficient degree to cause severe narrowing of the lumen. It is of interest that the lesions in Gartside's case, as in ours, were present at a site where sarcoidosis is common, although no submandibular gland tissue was seen in the specimen. Meyer et al. described a case[3] in which giant-cell granulomata in the lungs, lymph nodes, kidneys, and liver were associated with involvement of meningeal arteries and veins by sarcoid lesions. The vascular lesions in this case were different from those in Gartside's and in our cases in that they were situated principally in the perivascular spaces and adventitia and the elastica and media were not affected except in rare instances in which the entire wall of the small artery was undergoing acute necrosis.

There are some points of resemblance between the tissue reactions in our two cases of sarcoidosis with arterial changes and forms of polyarteritis nodosa and its related condition, giant-cell arteritis. The latter is a granulomatous inflammation seen most commonly in the temporal artery and predominantly affecting the media, with associated rupture of the internal elastic lamina and intimal thickening. Many other large arteries may be affected; unlike our two cases the vessel walls alone are involved, no tubercles being found in the surrounding tissues.[4] In some cases of polyarteritis nodosa a granuloma with Langhans and foreign-body giant cells may be present in the vessel wall, and the appearances are then similar to those of temporal arteritis[5]; cases of polyarteritis nodosa may also show lesions outside the blood vessels, notably in the spleen,[6] with a structure similar to that of the sarcoid granuloma. Jaques described a case in which typical sarcoid lesions were present in the lungs and the liver, while vessels in the kidneys showed the appearance of necrotizing arteriolitis.[7] Fibrinoid necrosis, although not seen in our cases, is quite commonly seen at the center of sarcoid tubercles.

A commonly held view of the etiology of polyarteritis nodosa is that it represents a hypersensitivity reaction to a foreign protein. Evidence has been put forward in support of a similar view in the case of sarcoidosis.[8] Eosinophilia has been noted, and an increase of serum globulins is common in sarcoidosis.[9] Teilum drew attention to precipitates of a homogeneous substance in the reticuloendothelial system in cases of sarcoidosis which ultimately become hyaline tissue (paramyloidosis).[10] Found commonly in the spleen, these lesions resemble the periarterial fibrosis seen in the spleen in disseminated lupus erythematosus, another condition with histological features related to polyarteritis nodosa, and likewise possessing a possibly allergic basis. Teilum relates sarcoidosis, disseminated lupus erythematosus, and other conditions with a high blood globulin as forms of "allergic hyperglobulinosis" determined by persistent stimulation of immune mechanisms. We consider that the presence of granulomatous arterial changes in our two cases of otherwise typical sarcoidosis is further evidence of the relationship of sarcoidosis to polyarteritis nodosa and other conditions with a probable hypersensitivity basis.

There is experimental evidence suggesting that sarcoidosis may have an allergic basis and linking it with polyarteritis nodosa. Germuth followed the changes in rabbits receiving a single dose of crystallized bovine albumin both histologically and by serial estimation of the antigen and its antibody. Among other changes, lesions of polyarteritis nodosa were produced in the vessels of many organs, and, in addition, granulomatous foci consisting of epithelioid and foreign-body giant cells were formed in the spleen and lymph nodes. These changes

developed during the phase of antigen elimination and regressed after the antigen had disappeared and free antibody was present.[11] Heptinstall and Germuth showed that prolonged sensitization produced by injection of repeated large doses of protein antigen into rabbits caused granulomatous lesions in the walls of renal arteries.[12] Thus a lesion resembling the common tissue form of sarcoidosis and an arterial lesion resembling that found in the two cases here described, as well as changes comparable to those of classical polyarteritis nodosa, may be elicited by the production of experimental hypersensitivity in rabbits.

The association of bronchiectasis with sarcoidosis seen in Case 1 is very unusual. Either condition can be postulated as having led to the development of the other. There are a few reports of pulmonary cavitation or bronchiolar dilatation in sarcoidosis,[13] but in these cases there was marked fibrosis, due to the healing of sarcoid lesions. In our case there was only a small amount of fibrosis, which is insufficient to account for the striking degree of bronchial dilatation. Another way in which sarcoid lesions might have produced bronchiectasis is by narrowing and obstructing the lumina of the bronchi. Macfarlane and Sommerville have suggested that the bronchiectasis of children may be related to lymphoid hyperplasia of bronchial walls due to infection with adenovirus.[14] Citron and Scadding described stenosis of bronchi due to sarcoid lesions in their walls, with dilatation distal to the stenosed areas.[15] In our case, however, there was no radiological evidence of major bronchial stenosis. It seems more likely that the sarcoid-like lesions represent a secondary process occurring in a lobe which was already bronchiectatic. In Symmers' Case 5, where obstruction of a main upper lobe bronchus by a bronchial adenoma had led to bronchiectasis and bronchiolectasis, tuberculoid foci were present in the pulmonary lymphoid tissue.[16] Sarcoid-like lesions may occur as a complicating factor in torulosis and other fungus infections[17] and in cases of carcinoma.[16]

Summary

Two cases of pulmonary sarcoidosis are described which show, in addition to the typical changes of this condition, giant-cell granulomatous lesions in branches of the pulmonary artery. The arterial changes are in the media or intima and are associated with intimal thickening and rupture of the internal elastic lamina.

The presence of these lesions suggests a relationship between sarcoidosis and polyarteritis nodosa. The possibility of sarcoidosis being a hypersensitivity state is discussed.

Case 1 showed also severe bronchiectasis; it is thought that this was the initial lesion which may have predisposed to the development of the sarcoidosis.

Addendum

Since this paper was submitted for publication, an additional case showing the changes described has been published.[18] The patient was a 55-year-old woman who died in an acute asthmatic attack. At autopsy sarcoid-like granulomatous lesions were seen in the lungs, heart, kidneys, mesenteric blood vessels, periadrenal vessels, and ganglia. The intima of pulmonary blood vessels was thickened and the internal elastic lamina ruptured, in association with foci of the granulomatous process. There were yellow granules in macrophages and giant cells, but, apart from this and the arterial changes, the appearances and distribution of the lesions were compatible with Boeck's sarcoidosis. This case would thus seem to fit into a morphological category similar to that of Gartside's and our cases.

Department of Pathology, St. Mary's Hospital.

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News and Comment

GENERAL NEWS

The George Howell Coleman Medal.—The George Howell Coleman Medal for 1959 of the Chicago Institute of Medicine was awarded to Dr. Edwin F. Hirsch for his many contributions to pathology and to medicine.

PERSONAL NEWS

Appointments of Dr. Gordon Ross Hennigar.—Dr. Gordon Ross Hennigar, Professor of Pathology, has been made Acting Chairman, Department of Pathology, State University of New York—Downstate Medical Center, and Acting Director of the Institute of Pathology, Kings County Hospital, during the sabbatical leave of Dr. Patrick J. Fitzgerald, Chairman, who is at the Department of Biochemistry, Oxford University, Oxford, England. Dr. Stanley M. Aronson, Professor of Pathology, has been appointed Associate Director of the Institute of Pathology.

ANNOUNCEMENTS

Course in Histochemistry.—A laboratory course in histochemistry will be offered June 6 to 18, 1960, at the University of Kansas Medical Center, Kansas City, Kan. Tuition will be $75 per week. Guest faculty will include Dr. Ned Feder, of Boston; Dr. George Glenner, of Bethesda, Md.; Dr. Frank Johnson, of Washington, D.C.; Dr. Marvin Nachlas, of Baltimore; Dr. Robert E. Stowell, of Washington, D.C., and Dr. Hisako Yokoyama, of Chicago. Applications should be addressed to the Department of Postgraduate Medical Education, University of Kansas School of Medicine, Kansas City 12, Kan.

The International Association of Gerontology.—The Fifth International Congress of Gerontology will be held in San Francisco, Aug. 7-12, 1960. Inquiries regarding this Congress should be addressed to Louis Kuplan, President, APO 2103, Sacramento 10, Calif.

DEATHS

Col. Robert L. Hullinghorst Dies.—Col. Robert L. Hullinghorst, Deputy Director of the Walter Reed Army Institute of Research, died in the Naval Hospital in Bethesda, Md., on Dec. 10, 1959, at the age of 43.

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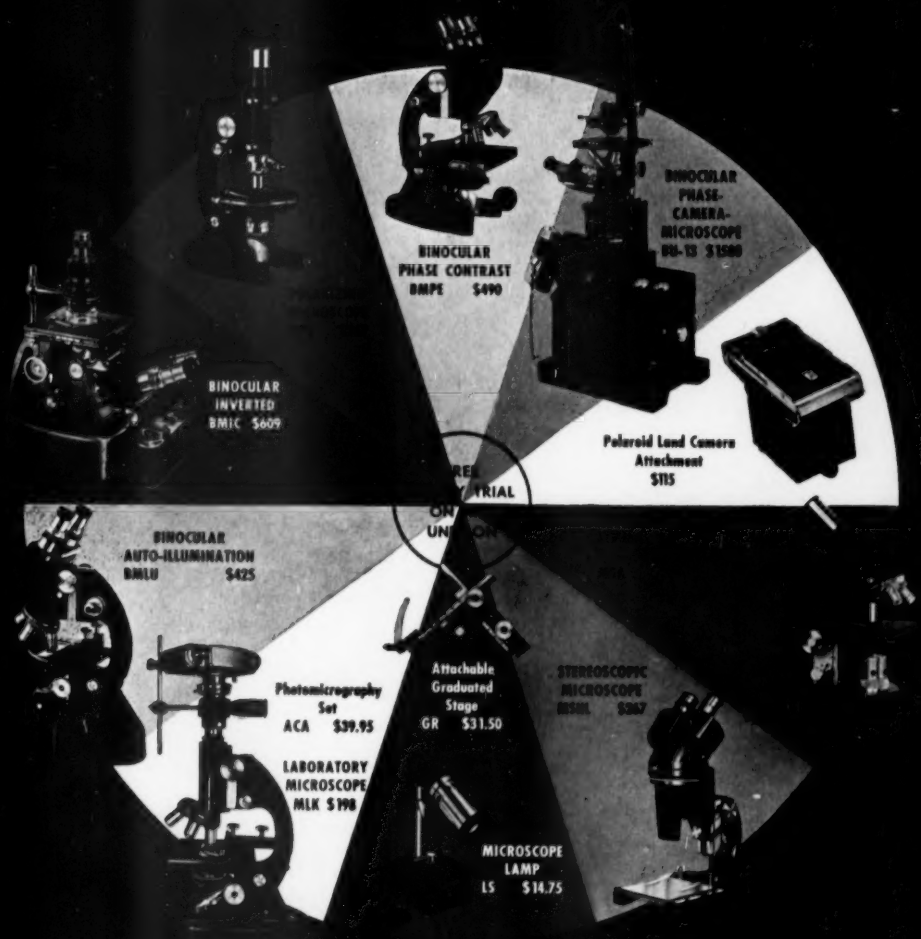

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All Paragon Tray Drawer Cabinets are manufactured in standard sizes so that any number of sections may be interlocked to form one cabinet to accommodate any number of varied slides. The dimensions of the different cabinets are the same as to length and width, varying only in height. The cabinet formed by interlocking may be 18¾ x 15¾; 18¾ x 11 or 18¾ x 5 or it may be a pyramid with the sections varying in width.

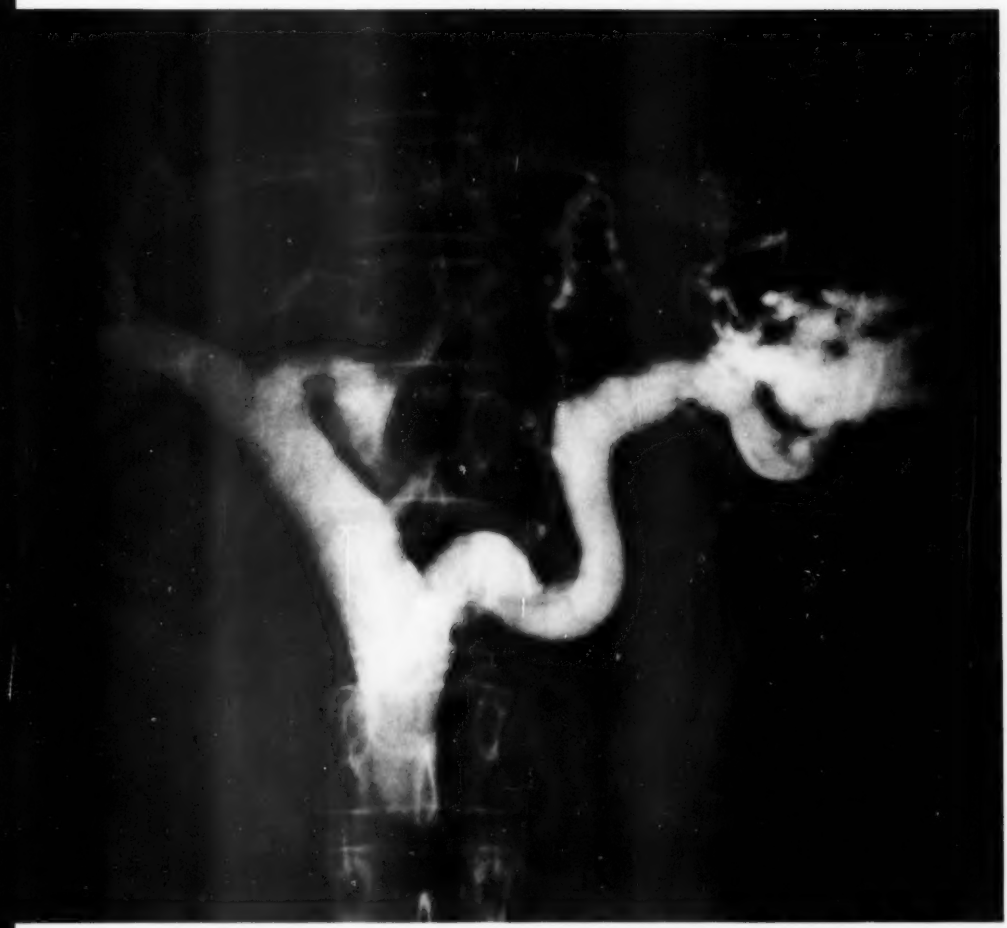
C221—Capacity 1500 Slides—18¾ x 11 x 3¾
For Filing KODACHROME TRANSPARENCIES and 2 x 2" SLIDES

SPECIFICATIONS: All Paragon Tray Drawer Cabinets are made of reinforced steel construction, olive green finish. Interlocking device enables several units to be joined into one. Each sectional unit contains removable drawers with hand grip in front and rear. Interlocking steel base obtainable whenever required. **Constructed according to rigid specifications—not merely adapted.**

Address your orders and inquiries to Dept. P.

Manufactured Exclusively by

PARAGON C. & C. CO., Inc. • 2540 Belmont Ave., New York 58, N.Y



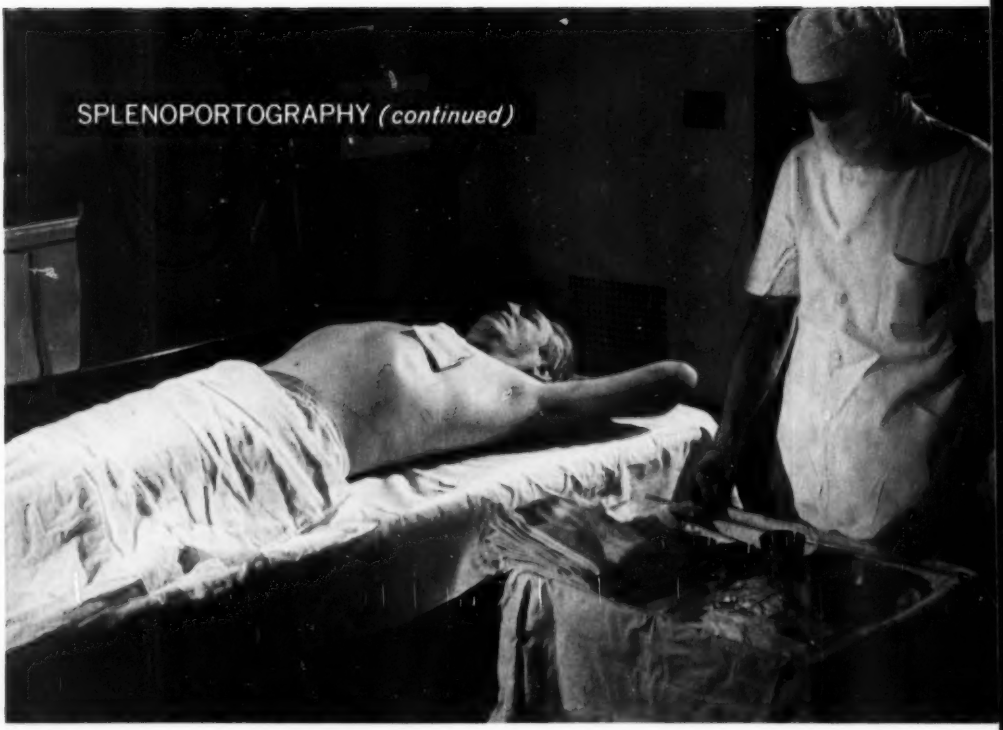
Splenoportogram (on Kodak Blue Brand Medical X-ray Film) of patient with cirrhosis of liver showing obstructed hepatic circulation and collateral veins including gastric and esophageal varices.

The technic of SPLENOPORTOGRAPHY:

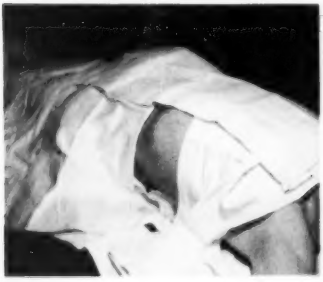
5 steps recorded on Ektachrome Film

People, long ago, had words for it . . . "One picture is better than a thousand words." True then; truer than ever today, when words and color illustrations may be combined to outline the steps in any technic or procedure. Surely, every physician who has a story to tell—procedures to describe—should investigate color photography. The cost of equipment and processing is small, the rewards great.

1. Equipment for splenic manometrics: Saline manometer; cups with contrast material, procaine, skin antiseptics and saline; syringes; connecting plastic tubes; stopcocks; needles; forceps.

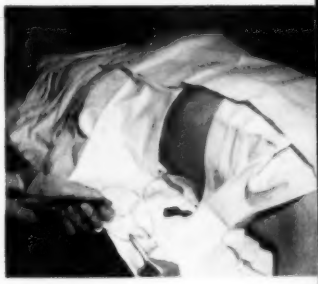


2. With patient in supine position on x-ray table, tube is centered over upper abdomen. Radiographs are made serially one per second during injection over a period o 15 seconds, after which they are obtained at 5-second intervals



3. Thin-wall, 16-gauge needle inserted through ninth intercostal space in midaxillary line has entered spleen. Local anesthesia used.

4. Pliable plastic tube connects saline manometer to needle. Splenic pulp pressures, corrected to the level of right atrium, indicate portal pressures.



5. Contrast material is being injected rapidly into splenic pulp as radiographs are obtained. (Figure 2).

New tips and tops on PYREX® pipets

. . . some of the new pipets we've added during the past year or so

You can be pickier than ever about the pipets you use for biological work; we've added to and refined the entire line of PYREX pipets.

New tips. There's a bigger hole in the tip of No. 7087 that lets you work faster with viscous liquids such as heavy creams, curds, and oils. Thanks to an extra-long, narrower tip on No. 7084, you can work more easily with Warburgs, small flasks and tubes, and the like. Tips on No. 7105 pipets, designed especially for Folin tests of urine and blood sera, are now uniform in length and diameter.

Like all PYREX tips these are stoned to a smooth bevel which reduces snagging and breakage significantly.

New tops. No. 7086 is an ACCU-RED pipet with a cotton plug top for safe transfer of a virus or pathogen; for the first time you can combine the strength and long-lasting marks of ACCU-RED with cotton plugs.

No. 7096 is a rinse-out pipet for accurate micro dilutions.

All our tops are flat and smooth for precise finger control. They're also fire polished for easy removal of lipstick and other stains.

There are many more special pipets, and old standbys, listed in your PYREX labware catalog LG-1 and supplements . . . along with the world's most complete line of lab glassware.

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